The Journal of Laboratory and Clinical Medicine

VOL. 21 DECEMBER, 1935 NO. 3

CLINICAL AND EXPERIMENTAL

THE BEHAVIOR OF THE EOSINOPHILES IN RHEUMATIC FEVER*

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ALTHOUGH the source and function of the eosinophilic polymorphonuclear leucocytes are not definitely known, certain characteristics of their behavior in the peripheral circulation during the course of infections have been described. It has been clearly established that quantitative diminution of the eosinophiles is found during the height of many febrile diseases, such as pneumonia, typhoid fever, erysipelas, acute appendicitis, etc.[1-7] Subsequently, in these infections, their number increases, frequently reaching a level higher than normal. In scarlet fever the hypoeosinophilia is of very short duration and hypereosinophilia appears considerably before defervescence. In acute infections, other than scarlet fever, the eosinophilic rhythm is (1) disappearance—fastigium, (2) reappearance—defervescence, (3) transient eosinophilia—convalescence. The presence of eosinophiles and eosinophilia during the course of acute febrile diseases are universally accepted as favorable prognostic signs.[1, 3, 4, 8-11]

In chronic infections the eosinophiles do not behave with the same regularity. For example, it has been shown by Bezançon, de Jong, and de Sorbonnes[12] that in tuberculosis, the often fatal acute pneumonic forms are accompanied by persistent aneosinophilia† or hypoeosinophilia, but in chronic tuberculosis with the usual exacerbations, the eosinophilic rhythm is similar to that in acute fevers with favorable prognosis, i.e., the hypoeosinophilia is succeeded by eosinophilia. There is, however, one important difference, viz.,

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†Aneosinophilia refers to the absence of eosinophiles in a routine count of 100 cells.

in nonspecific acute infections, the eosinophilia is comparatively short in duration, but in tuberculosis it frequently persists for weeks and sometimes even for months. It is seen not only at the end of acute episodes but also in the intervals between them.

Eosinophilia has already been described in rheumatic polyarthritis.[6-9, 13] Tuerck[8] and Schwarz[6] observed rising eosinophile counts as the inflammation in any one joint receded, and Tuerck also described convalescent eosinophilia as high as 13.8 per cent. Mayer[9] reported a case with constant eosinophilia in which the course was short and mild and the recovery was rapid. Wile[4] described an early eosinophilia in acute articular rheumatism and also in chorea. Page, Turner, and Wilson[13] found eosinophilia in rheumatic carditis as well as in polyarthritis and chorea. Perry[14] attached little significance to what he termed the usual slight postinfectious eosinophilia with lymphocytosis of acute rheumatic fever and carditis.

The eosinophilic response to rheumatic infection must be further considered in relation to the allergic hypothesis. Swift[15-17] has summarized the evidence pointing to the allergic nature of many rheumatic manifestations, but found eosinophilia in only three of the thirteen cases studied, all of whom had chorea. The relation of eosinophilia to the allergic state in general has long been suspected but its presence is not constant, and it has been impossible to attach specific import to it. In asthma, the eosinophiles are not invariably increased, but their number is more frequently high than in other allergic disturbances.[18] It has even been considered presumptive evidence of allergic etiology in the absence of positive skin tests.[19] Local eosinophilia accompanied by peripheral circulation eosinophilia is known to appear at the site of foreign protein injections.[20, 21] The nasal mucosa is also a site of eosinophile infiltration in asthma and hay fever.[22] Ringoen[23] produced eosinophilia in the peritoneal cavity and blood stream of sensitized animals in which the shock dose had produced an anaphylactic reaction. The linkage of hypersensitivity to eosinophilia is well illustrated in the case of scarlet fever. Not only is eosinophilia present in the early stages of this disease, but it has also been shown by Vaughan[24] that persons with positive Dick skin tests reacted in every case with a rise in eosinophiles to immunizing doses of the specific toxin. In two cases, both with strongly positive skin tests, the eosinophilia was coincident with the appearance of a rash. The same phenomenon has been noted by Kleinberger[7] in response to therapy with tuberculin. In contradistinction to scarlet fever and tuberculosis, where positive skin tests are valuable in indicating sensitivity to a specific organism, in rheumatic fever the value of positive skin tests is considerably diminished because of their inconstant and unpredictable appearance. In rheumatic fever an added difficulty presents itself, viz., no specific etiologic agent has been conclusively demonstrated. Therefore, although we may accept positive Dick tests, tuberculin sensitivity and eosinophilia, when they occur, as direct evidence of bacterial allergy in scarlet fever and tuberculosis, the corresponding phenomena occurring under similar conditions in rheumatic fever can only be accepted as additional indications of the probable presence and importance of hypersensitivity.

The short case histories presented below illustrate four features of the behavior of the eosinophiles during the course of rheumatic infection. These are (1) the disappearance of eosinophiles from the peripheral circulation during accessions of acute polyarthritis and carditis, (2) the reappearance of eosinophiles and eosinophilia during the early stages of recovery, (3) persistent eosinophilia in cases of continued activity, (4) transient hypo- or aneosinophilia occurring during minor exacerbations in cases of chronic rheumatic heart disease.

CASE 1.—Male, negro, aged forty-eight years, was admitted to the clinic in March, 1932, complaining of dyspnea, substernal pain, pain in the left shoulder, and cough. These symptoms began two weeks after an acute upper respiratory infection. He had had an attack of arthritis at the age of forty years in which the only joint affected was the left hip. One year later, he had migrating polyarthritis. For the next three years, there were minor joint pains and then a symptom-free interval succeeded finally by cardiac decompensation, which, interpreted in the light of later events, probably was due to rheumatic carditis. The anatomic lesion was rheumatic mitral and aortic endocarditis. Auriculoventricular conduction was delayed (0.24 sec.). He improved slowly and in December, 1932, he was able to resume part-time work. During the remainder of the period of observation, rheumatic activity was considered to be subsiding, but there were transient, mild exacerbations. The prolonged a-v conduction time remained unchanged.

Table I illustrates the behavior of the eosinophiles in the peripheral circulation. The period of slow clinical improvement was marked by normal and elevated eosinophile counts. The single instance of aneosinophilia was found during a period when signs of increased cardiac embarrassment had temporarily reappeared. This case illustrates the disappearance of the eosinophiles in the routine blood count (100 cells) during exacerbations of acute rheumatic carditis and their reappearance during stages of recovery. It also illustrates the increase of the eosinophiles to a level considerably above normal (8 per cent) during the stage of slowly subsiding active infection, and aneosinophilia during an intercurrent minor exacerbation.

TABLE I

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 1

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.	BASO.
9/23/32	11,300	71	1	27	0	0	0
10/ 7/32	7,300	70	0	26	3	0	0
12/16/32	5,700	50	0	40	6	2	2
1/13/33	8,000	54	10	26	6	2	2
1/27/33	6,300	52	0	38	4	6	0
2/24/33	7,000	64	2	26	2	6	0
5/19/33	7,300	54	2	34	8	2	0
6/ 7/33	6,400	58	0	34	6	2	0
7/14/33	7,100	46	6	36	9	3	0
8/ 9/33	7,100	64	12	18	6	0	0
9/25/33	6,300	56	8	32	0	4	0
10/ 6/33	5,950	49	6	38	5	2	0
11/ 3/33	6,300	44	4	38	6	8	0

CASE 2.—White male, thirty-one years old, a blacksmith, was admitted to the hospital May 4, 1934, with acute polyarthritis which involved successively the right knee, ankles, and the wrists. He had fever, leucocytosis, a high percentage of immature polymorphonuclear leucocytes, and increased sedimentation rate.

Defervescence with disappearance of the joint symptoms began a week after admission. The gradual recovery from this infection is evidenced in Table II by the fall in temperature and pulse rate, and best by the gradual decline of the sedimentation rate. The latter shows a gradual decrease from 29 mm. to 4 mm. in six weeks (Cutler method, one-half hour).

These signs were accompanied by gradual clinical improvement and at first by the transient reappearance in the peripheral blood of eosinophiles in small numbers. During the period of established convalescence eosinophiles were constantly present, rising to a slightly increased level of 5 per cent, with the exception of one observation when they were absent. The latter count was made when a rise in temperature, pulse rate, leucocyte count, and immature forms indicated a recrudescence of infection. There was a complete return to normal two days later, all indices again declining, and the eosinophiles reappeared.

TABLE II

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 2

DATE	W.B.C.	SEG-MENTED	BAND	LYMPH.	MONO.	EOS.	BASO.	COMMENT TEMP.	COMMENT PULSE	SED. TIME* MM.
5/ 4/34	10,200	58	10	24	6	2		102.0-103.4	96-102	
5/ 9/34	15,600	66	19	9	6	0		101.8-102.6	88- 96	29
5/10/34	13,450	62	16	18	4	0		101.0-102.8	84- 96	
5/10/34	12,800	60	14	24	1	0	1	100.0-102.0	72- 86	
	12,650	52	15	26	4	2	1			
	11,900	52	19	20	8	1				
5/12/34	12,700	49	18	26	7	0		100.8-101.6	70- 84	
5/14/34	13,100	43	16	31	10	0		99.8-101.0	68- 90	
5/17/34	12,800	44	18	32	6	0		99.0- 99.6	68- 76	25
5/21/34	13,000	61	11	20	6	2		98.4-100.0	70- 88	17
5/25/34	7,200	59	4	31	6	0		99.0- 99.6	80- 86	
5/28/34	8,450	46	2	40	8	4		98.4- 99.4	80- 84	17
5/31/34	6,800	46	2	45	3	4		98.6- 99.6	80- 84	13
6/ 4/34	7,100	56	2	38	2	2		98.8- 99.0	74- 80	10
6/ 7/34	6,900	43	6	47	3	1		98.6- 99.4	82- 90	
6/11/34	9,700	54	4	33	4	5		98.8- 99.6	82- 84	5
6/14/34	11,100	62	8	24	6	0		98.6-100.6	80-108	
6/16/34	7,200	57	4	30	8	1		98.8- 99.4	78- 84	5
6/18/34	8,900	51	2	34	11	2		98.6- 99.4	76- 84	4

*Cutler method, one-half-hour rate.

This case illustrates the reciprocal relation between the indices of infection and the disappearance and reappearance of eosinophiles in a case of acute rheumatic fever with predominant polyarthritis.

CASE 3.—Female, aged thirteen years. This patient came under observation with residual joint pains and a mitral systolic murmur following an attack of acute polyarthritis which had involved many joints successively one month before admission. During the one month which she spent in the hospital she became afebrile and the leucocyte count fell from 14,700 to 9,800 (Table III). The presence of eosinophiles and occasional slight eosinophilia accompanied a steady clinical improvement and fall in the total white cell count. Aneosinophilia, occurring once, was accompanied by a slight leucocytosis. At discharge, there were

TABLE III

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 3

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.
3/19/34	14,700	61	4	31	3	1
3/31/34	13,200	46	4	41	4	5
4/ 2/34	11,100	49	3	43	2	3
4/ 6/34	10,500	60	6	30	2	2
4/10/34	10,000	46	4	42	2	6
4/12/34	11,700	54	4	36	4	2
4/14/34	10,700	44	5	49	2	0
4/18/34	9,800	41	9	44	3	3
6/20/34	10,200	43	6	47	4	0
7/ 5/34	11,400	46	4	47	3	0
10/10/34	9,500	62	3	28	5	2

3 per cent eosinophiles. The aneosinophilia in the two subsequent follow-up examinations was coincident with an increase in the leucocyte count to 11,400.

CASE 4.—Female, admitted at the age of eight years for an eczematoid skin eruption, was discovered to have mitral endocarditis during a routine physical examination. There had been transient muscular pains in the legs for the two preceding years. Three years later she again came to the clinic because of pain in the legs. From that time to the present, she has shown continuous active rheumatic infection with dyspnea, palpitation, tachycardia, fever, and hemorrhagic spots in the mucous membranes of the mouth and of the skin.[25] In the more recent observations, there has been some symptomatic improvement, and for the first time in two years, the cardiac rate has fallen below 100 per minute.

This case illustrates a recurrent eosinophilia during the course of continuous rheumatic activity of moderate severity. At no time was aneosinophila or hypoeosinophilia obtained in eleven observations over a period of thirteen months. Although tachycardia was always present and fever at four examinations, the patient's subjective condition was excellent. She always insisted that she felt "fine" even when her pulse rate was 144. The percentage of polymorphonuclear leucocytes was never higher than 62 per cent and immature forms were only increased slightly. It should also be noted that slight monocytosis was present on three occasions. A peak of 14 per cent eosinophiles was found at the last examination, at which time her clinical condition was strikingly good.

The continued presence of eosinophiles with frequent eosinophilia of unusual degree, characterized a case of rheumatic endocarditis in which activity was continuous but not disabling. The absence of leucocytosis and the mild monocytosis confirm the impression of a mild chronic infection and a comparatively good immediate prognosis. This patient may be thought of as being in the recovery stage for thirteen months.

TABLE IV

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 4

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.	BASO.	TEMP.	PULSE
10/ 7/32	10,000	55	6	27	8	4		101.0	116
11/ 4/32	6,200	39	6	49	4	2		100.6	108
12/ 2/32	9,800	48	9	27	6	9	1	99.0	112
1/13/33	8,600	43	5	33	11	8		100.2	144
2/10/33	9,000	42	10	40	4	4		100.6	116
3/10/33	8,400	53	5	28	10	4		99.0	124
4/ 7/33	9,650	41	5	36	10	8		99.6	100
5/18/33	9,700	56	6	30	6	2		98.6	116
7/28/33	11,100	39	4	42	6	9		99.4	100
10/20/33	9,950	51	9	28	8	3	1	99.4	120
11/17/33	9,050	60	1	20	4	14	1	99.0	92

CASE 5.—A white female, fifty-two years of age, admitted to the hospital with acute polyarthritis of one month's duration and mitral stenosis and insufficiency. She had had polyarthritis at five years and thirty-five years. The symptoms were dyspnea, palpitation and slight dependent edema for two weeks prior to admission. Auricular fibrillation was present on the first day, after which it was replaced by normal sinus rhythm. Transient fibrillation appeared once more during hospitalization. Fever and tachycardia were present throughout, with remissions and exacerbations and a pleuropericardial rub appeared for a few days. She also had transient partial a-v block.

The record of the blood findings (Table V) illustrates a complete disappearance of the eosinophiles from the blood during the height of the polyarthritis attack. Reappearance of eosinophiles accompanied her first temporary improvement when joint involvement had entirely disappeared. The eosinophiles again disappeared and remained absent during the exacerbated activity of the rheumatic infection of the heart. The last three blood counts showed normal eosinophile counts together with a marked drop in the polymorphonuclear cells and a rise in the monocytes.

CASE 6.—Male, white, thirty-two years of age, admitted to the clinic Jan. 13, 1931, complaining of shortness of breath, pain in the pericordium and left knee joint, and the arms

TABLE V

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 5

DATE	W.B.C.	SEG-MENTED	BAND	LYMPH.	MONO.	EOS.	TEMP.	PULSE	SED. TIME MM.
5/ 9/34	8,600	70	8	18	4	0	99.8-101.4	92-100	14
5/14/34	8,600	58	10	20	12	0	100.0-101.4	88- 94	25
5/15/34	10,200	53	13	26	8	0	99.6-101.8	90-100	
5/18/34	6,900	61	16	19	4	0	99.0-100.4	84- 90	12
5/21/34	8,950	67	14	15	3	1	98.4-100.6	84- 92	6
5/28/34	7,400	66	10	18	6	0	99.8-101.0	88- 98	7
5/31/34	7,100	70	10	12	6	2	99.6	86- 88	10
6/ 4/34	8,300	68	10	12	6	4	99.4-102.0	84- 88	18
6/ 7/34	6,600	70	11	12	6	1	99.4-100.0	84- 88	20
6/11/34	5,900	61	12	16	9	0	99.8-100.6	84- 88	15
6/15/34	8,500	62	8	20	10	0	99.4-100.8	82-100	11
6/18/34	11,100	65	16	14	5	0	100.2-100.6	82- 84	19
6/21/34	5,400	64	12	20	4	0	98.6- 99.6	68- 84	25
6/25/34	4,900	54	10	24	10	2	98.8- 99.2	84- 88	10
6/28/34	5,650	46	11	31	10	2	98.8- 99.0	78- 80	13
6/30/34	5,950	40	12	36	10	2	98.8- 99.2	84- 96	18

and soles of the feet. There was no history of antecedent rheumatic infection. Within two years before admission, he had been in hospitals twice for partial decompensations. The anatomic diagnosis was mitral endocarditis.

The blood examinations from January, 1931, to April, 1932, revealed frequent leucocytosis, a relatively high polymorphonuclear count and a high percentage of immature forms. Of eight counts made during this period, eosinophiles were absent five times. After May, 1932, eosinophiles were found in every film examined, reaching 6 per cent on four occasions. Concomitantly, the polymorphonuclear and immature forms maintained a decidedly lower level than during the preceding years. It is considered that the changes in the blood count illustrate a case of very mild rheumatic activity gradually decreasing and reaching a minimal level.

TABLE VI

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 6

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.	BASO.
1/15/31	11,300	63	2	22	3	3	0
1/21/31	12,300	62	8	23	3	0	4
1/21/31	8,900	64	9	21	0	3	3
1/22/31	12,400	63	7	24	7	0	2
1/29/31	11,800	60	8	29	2	0	1
11/10/31	8,200	42	10	42	4	2	0
4/14/32	8,200	62	2	30	6	0	0
5/30/32	12,100	52	10	36	2	0	0
9/23/32	8,500	48	3	42	6	1	0
10/21/32	11,600	51	8	32	8	1	0
11/18/32	13,600	52	4	34	6	4	0
1/ 4/33	9,000	62	2	18	10	6	2
2/17/33	11,000	52	4	36	6	2	0
3/24/33	9,000	50	8	28	8	6	0
6/ 2/33	10,250	46	4	38	8	2	2
8/ 1/33	9,350	52	4	38	2	4	0
8/15/33	8,300	53	6	36	4	1	0
11/17/33	12,650	61	8	20	4	6	1
12/29/33	10,600	48	2	41	3	6	0

CASE 7.—Male, admitted to the hospital in December, 1928, at the age of fifteen years for his second attack of polyarthritis within ten months. Mitral and aortic endocarditis were present. At the end of six weeks the acute articular symptoms and the fever had subsided,

and he was discharged to the clinic. Slight exacerbations of rheumatic activity appeared in February and again in April, 1929. In each of these there was fever but no joint pain. In the summer of 1929, he improved but not until late that year was he allowed to resume his school work. Following this there were short bouts of infection in December, 1929, and in October, 1930. In the interim between these two attacks there were no symptoms. In January, 1933, he had another short bout of fever without symptoms, other than slight malaise. He returned to work and since then has had no signs of renewed infection.

TABLE VII

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 7

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.	BASO.
9/26/29	13,100						
10/10/29	9,600	50	5	35	7	3	
10/15/29	9,200	52	4	40	2	2	
10/29/29	10,400	48	0	38	10	3	1
10/30/29	6,400	50	2	42	3	3	
6/20/30	9,400	58	2	35	3	2	
10/ 9/30	11,150	40	6	52	2	0	
10/21/30	9,400						
10/25/30	9,800						
11 /9/31	12,500	46	6	42	2	4	
11/17/31	9,200	50	0	44	2	4	
1/21/32	8,900						
4/25/32	6,850						
12/ 3/32	8,800	46	2	36	12	4	
5/19/33	12,700	52	4	34	8	2	
6/30/33	9,000	63	6	20	6	4	1
9/ 8/33	8,700	54	3	39	2	2	
10/ 6/33	8,050	51	4	42	1	2	
1/26/34	8,500	41	4	38	10	7	

This case is marked by the almost continuous presence of the eosinophiles in the blood. One observation of aneosinophilia was made. The patient was always relatively in good condition throughout the period of observation. Eosinophilia of very mild degree occurred several times reaching a level of 7 per cent at the last examination.

DISCUSSION

The behavior of the eosinophilic polymorphonuclear leucocytes in rheumatic fever is similar in all respects to that seen in other infections. In acute rheumatic polyarthritis and carditis during the fastigium, there is hypo- or aneosinophilia. This is succeeded by a return of the eosinophiles to the blood with frequent convalescent eosinophilia. The greatest variety of eosinophile response is seen in patients who have well-developed rheumatic heart disease. In these subjects, there are found hypoeosinophilia, aneosinophilia and persistent or recurrent eosinophilia. From a study of the above tabulated differential counts, however, it is apparent that in so-called chronic rheumatic heart disease, the presence and absence of eosinophiles and the occurrence of eosinophilia have the same significance as in other infections. The concept of rheumatic heart disease as a chronic infection with frequent acute exacerbations of variable degree offers the key to an understanding of the apparent complexity of the eosinophilic behavior. Thus repeated and continuous absence of eosinophiles from the blood over a period of time was always found to be associated with other evidences of a severe and active infection. In the majority of cases showing continuous aneosinophilia or hypoeosinophilia of

this type, the patient was ill enough to be confined to bed. Conversely, when the eosinophiles were continuously present in normal or increased numbers, the infection was always subsiding. It may be stated that eosinophilia always indicates convalescence, which in rheumatic fever is admittedly very often a protracted process. Finally, occasional aneosinophilia occurring in patients who usually present normal or high counts, undoubtedly indicates miniature exacerbations of activity which give rise to little or no clinical disturbance.

Swift has shown the close resemblance of rheumatic fever to tuberculosis and has pointed out many similarities in the natural history of the two diseases. The close correspondence of the eosinophile behavior in rheumatic fever with that described by the French observers in tuberculosis,[12] affords another point of identity and the analogy constitutes further evidence in favor of the allergic hypothesis.

Continued observation of the behavior of the eosinophiles, over long periods of time in the less acute cases, is a reliable index of activity of rheumatic infection and is valuable as a basis, in some cases, for immediate prognosis and clinical management.

CONCLUSIONS

A study of the eosinophiles in acute rheumatic polyarthritis and acute and chronic rheumatic heart disease leads to the following conclusions:

1. In acute rheumatic polyarthritis and carditis, the eosinophiles are absent or diminished in number during the height of the disease.

2. During the stage of recovery, the eosinophiles reappear and there is postinfectious eosinophilia as in other acute infections.

3. Continuous aneosinophilia and hypoeosinophilia over long periods of time occurring in all three types of the disease and their combinations, indicate intense activity of infection.

4. Recurrent and continuous eosinophilia indicate convalescence.

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THE INFLUENCE OF SUCROSE INGESTION ON AMINO ACID NITROGEN AND UREA NITROGEN CONCENTRATION OF THE BLOOD*

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WHILE the metabolism of glucose has been subjected to intensive study less attention has been given to that of sucrose. An investigation concerning the influence of alimentary dextrose upon the amino acid nitrogen and urea nitrogen content of the blood has been reported in a recent paper in this journal.

By the use of similar experimental methods 26 patients have been subjected to sucrose tolerance tests and the corresponding blood sugar, amino acid nitrogen, and urea nitrogen was determined at the fasting, one-hour, two-hour, and three-hour intervals following the administration of the sugar.

Analysis of the data in Table I demonstrates the fact that alimentary sucrose also depresses the postabsorptive level of amino acid nitrogen and urea nitrogen. In general, the results are similar to those secured with glucose. Thus the average maximum decrease in amino acid nitrogen re-

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Received for publication, March 13, 1935.

TABLE 1

THE INFLUENCE OF ALIMENTARY SUCROSE ON THE AMINO ACID NITROGEN AND UREA NITROGEN CONTENT OF THE BLOOD OF PATIENTS

CASE NO.	BLOOD SUGAR MG./100 C.C.				URINE AMINO ACID N. MG./100 C.C.					UREA N. MG./100 C.C.				DIAGNOSIS
	F.	1 HR.	2 HR.	3 HR.	SUGAR	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.	3 HR.	
1	105	130	99	99	Neg.	7.0	6.7	6.8	6.3	11.5	10.6	10.2	9.7	Pluriglandular endocrine disorder
2	100	145	111	100	Neg.	5.6	5.4	5.3	5.2	5.9	5.7	5.5	5.0	Diffuse colloid goiter; syphilis
3	95	98	103	91	Neg.	6.8	6.7		7.0	21.0	19.7	20.0	18.2	Syphilitic cardiovascular disease; aortic insufficiency
4	100	125	111	91	Neg.	6.7	6.1	5.8	6.0	12.5	11.5	10.7	10.9	Idiopathic epilepsy
5	94	148	103	81	Neg.	5.9	5.6	5.4	5.4	8.1	7.8	7.0	7.2	Syphilis; dental caries
6	91	118	74	78	Neg.	5.2	5.2	5.2	5.1	7.5	5.9	5.5	6.0	Pylorospasm
7	115	179	114	95	Neg.	6.5	5.8	5.8	5.6	8.0	8.5	7.5	6.9	Cellulitis, left leg
8	98	160	136	105	Neg.	6.6	6.3	6.3	5.8	6.5	6.1	5.6	5.3	Hypertensive cardiovascular disease
9	100	139	83	87	Neg.	6.7	6.7	6.1	6.2	16.7	16.2	15.5	14.5	Left hemianesthesia
10	100	137	95	105	Neg.	5.6	5.5	5.3	4.9	9.1	9.1	8.9	9.1	
11	95	95	91	98	Neg.	5.6	5.4	5.0	4.9	6.3	5.6	5.5	5.2	Bronchitis; toxic erythemia
12	100	157	145	133	Neg.					13.3	13.4	12.4	11.3	Hallux valgus deformity; lymphangitis
13	95	154	112	95	Neg.	6.0	6.0	5.7	5.7	18.8	16.7	16.7	16.5	Acute infectious arthritis, right hip
14	88	95	96	91	Tr.	6.6	6.1	5.7	5.7	13.7	13.2	12.6	12.4	Lymphangitis; infectious arthritis of knee
15	100	133	133	130	Neg.	6.2	5.7	5.9	5.7	10.0	9.1	8.5	8.2	Chronic multiple arthritis; infected tonsils
16	95	98	103	91	Neg.	6.8	6.7		7.0	20.9	19.7	20.0	18.9	Syphilitic cardiovascular disease; nephritis
17	100	200	204	147	Neg.	7.2	6.8	6.5	6.5	20.6	20.0	18.8	18.0	Tertiary syphilis; hypertensive cardiovascular disease
18	100	167	208	184	Neg.	5.2	5.2	4.5	4.8	17.7	18.8	17.2	16.8	Tenosynovitis right shoulder; otitis media
19	105	190	133	91	Neg.	6.1	5.9	5.8	5.4	6.0	5.9	6.3	5.6	Typhoid fever; syphilis
20	100	175	142	136	+	6.5	6.1	5.8	6.1	11.7	10.7	9.9	10.1	Chronic multiple arthritis; goiter; caries
21	97	163	182	142	Neg.	6.6	6.1	6.0	6.4	7.5	7.2	7.5	7.1	Toxic polyneuritis; hyperkeratosis of the feet
22	108	235	167	145	Neg.	7.0	6.4	6.4	6.0	10.7	10.0	8.9	8.8	Arthritis; hypertensive cardiovascular disease
23	91	200	192	167	Neg.	5.2	5.2	5.0	5.0	8.8	8.7	7.8	7.9	Multiple infectious arthritis; infected tonsil
24	91	222	142	80	Neg.	5.1	4.8	4.8	5.0	8.7	8.7	8.0	7.5	Acute pulmonary tuberculosis; pleurisy
25	118	238	222	187	++	7.0	6.5	7.0	7.1	12.5	11.6	11.5	12.5	Diabetes mellitus; bilateral cataracts
26	118	235	159	99	++	5.3	5.0	4.8	4.9	16.3	16.0	14.4	15.3	Diabetes mellitus; arteriosclerotic cardiovascular disease
Av.	99.9	159	132	117.2		6.2	5.9	5.7	5.75	11.9	11.4	10.86	10.95	
Av. maximum decrease (per cent)									8.07				8.74	

sulting from the ingestion of sucrose was 8.07 per cent as compared with a 11.78 per cent decrease in the same constituent produced by a similar quantity of dextrose. The average maximum decrease in urea nitrogen was 8.74 per cent as compared with a 13.04 per cent decrease with glucose. Thus alimentary sucrose seems to produce a somewhat smaller reduction in these nitrogen constituents of the blood than does an equivalent quantity of dextrose.

ON CHRONIC HYPERTENSION OF NERVOUS ORIGIN*

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EVIDENCE which has been accumulating within the last ten years has definitely proved that blood pressure is self-regulated by a reflex apparatus governed by impulses taking origin in special pressure receptors. These are located (Fig. 1) in definite areas of the arterial system: the walls of the aorta, close to the heart, and the two swellings at the beginning of each of the two internal carotid arteries, known as the *carotid sinuses* (Fig. 2.).

In the regions where receptors are located, the arterial walls are thinner and have a peculiar structure (fewer muscle fibers and more numerous elastic fibers) which make them more susceptible to changes in pressure. Deep in their adventitia they show rich nervous branchings which are connected with the pressure receptors. These, as shown by De Castro (1928), either keep a parallel direction to the very regularly arranged fibers of collagen (Fig. 3) or form spirals among them. Both dispositions predispose to change of form or position following changes in the degree of distention of the arterial walls.

Two nerves arise from the aortic receptors, the *aortic nerves*, whose development and distribution are better understood since Tello (1924) studied them. Another pair of nerves, the *carotid sinus nerves*, originate in the carotid sinus receptors and enter into the glossopharyngeus as it emerges from the skull.

By faradizing the central end of aortic or carotid sinus nerves, reflex heart retardation and fall of blood pressure are evoked.†

But what is more important than the results of artificially stimulating these nerves, is to know what are the effects exerted by the natural stimuli, playing continually upon the receptors from which they originate, i. e., by changes in arterial blood pressure. These have been demonstrated by different methods:

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Received for publication, December 21, 1934.

An address delivered to the assembly of the National Academy of Medicine of Mexico and the Texas Internists Club as guests of the Mexican Society for Internal Medicine in Mexico, D.F., September 19, 1934.

†For this reason, the name "depressor nerve," originated to designate the aortic nerves when these were the only known, can no longer be used specifically for them.

A. By first clamping the middle part of the common carotid, to suppress distention of the carotid sinus walls, and then unclamping the artery to have the sinus walls suddenly distended by the blood rushing in (Hering, 1927).

B. By submitting the carotid sinus, isolated from its normal circulation, to pressure changes in a perfusing fluid [Ringer fluid (Koch, 1929); defibrinated blood (Heymans-Bouckaert, 1929); blood from another animal (Heymans, 1929)].

C. By comparing the action of aortic and carotid sinus nerves, upon the height and shape reached by the blood pressure curve when the general blood

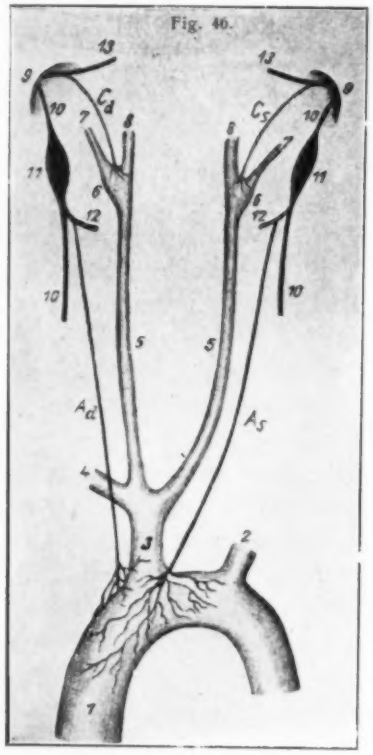

Fig. 1.

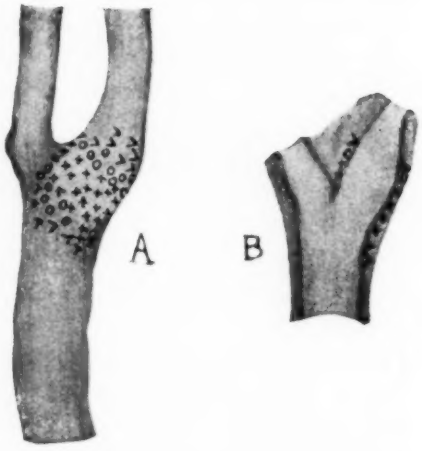

Fig. 2.

Fig. 1.—A sketch of the areas where the pressure receptors are located and the presso-sensible nerves originate. A_d and A_s, right and left aortic nerves; C_d and C_s, right and left carotid branches of glossopharyngeus; *6*, carotid sinus; *7*, internal carotid; *9*, *foramen jugulare*; *10*, vagus nerve, with *11*, its *ganglion nodosum* and *12*, superior laryngeal nerve; *13*, glossopharyngeus. (From Koch, 1929)

Fig. 2.—Human common carotid, with the carotid sinus at the origin of its internal branch. The number of receptors in the walls is maximal in the regions marked with + and lesser in those with > or 0. (From De Castro, 1928)

pressure is raised by stimulation of the peripheral end of the splanchnic nerves, by a constant faradic stimulus. First, when aortic and carotid sinus nerves are intact and free to come into play, and second, with these nerves partially or totally eliminated (Izquierdo, 1930).

Results yielded by all these methods coincide to demonstrate: (1) that blood pressure changes upward as well as downward in the aorta and sinus carotidus produce, through reflexes started in the aforesaid receptors, opposite

changes in the general blood pressure; (2) that the reflexes thus evoked (see Fig. 4) are mediated, mainly through changes in the vagal cardioinhibitory tonus and the general vasoconstrictor tonus, and secondarily upon the general vasomotor tonus and the cardioacceleratory tonus.

By means of these cardiac and vascular reflexes, both the *aortic and carotid sinus nerves maintain cardiac activity and blood pressure within limits of great constancy.* Under normal conditions their antagonizing effects may be as great as to counterbalance the general rise of blood pressure evoked by splanchnic stimulation (Fig. 5, *A*).

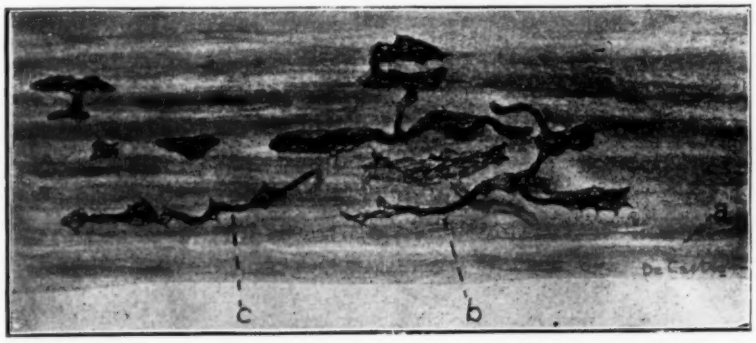

Fig. 3.—The sensitive receptors (*b* and *c*) from which the carotid sinus nerves are issued; *a*, elastic layer lying deeply in the adventitia. (From De Castro, 1928)

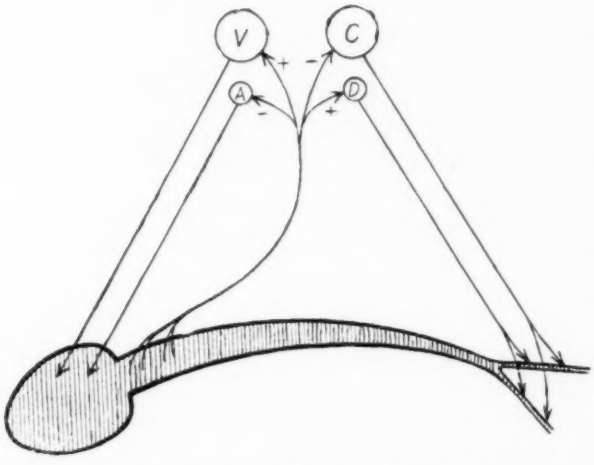

Fig. 4.—A sketch of the circulatory reflexes originating in the pressure receptors. *V*, vagus nerve; *C*, vasoconstrictors; *D*, vasodilators; +, excitation; −, inhibition. (From Koch, 1931)

When the aortic nerves have been previously severed and the carotid sinus nerves have been functionally excluded by clamping of the two common carotids, the blood pressure rises and the heart beats are at a higher rate (Fig. 5, *B*). But if then the splanchnic nerve is stimulated under the same conditions as before, the blood pressure curve is considerably higher than when both sets of nerves are intact (Fig. 5, *B*). This is because vasoconstric-

tion is left free to come into play without being antagonized by the compensatory reflexes.

The self-regulation exerted by this apparatus gains in accuracy on account of its being formed by four units with their origins located in different parts of the arterial system. When one of these units becomes activated, the others react at once to counterbalance the reactions produced by the former. This antagonistic action of the other units made it at first difficult to demonstrate the different tonus effects individually exerted by each of them.

Readers interested in the development and details of the problem are referred to three excellent monographs, written by Hering (1927), by Koch (1931), and by Heymans and collaborators (1932).

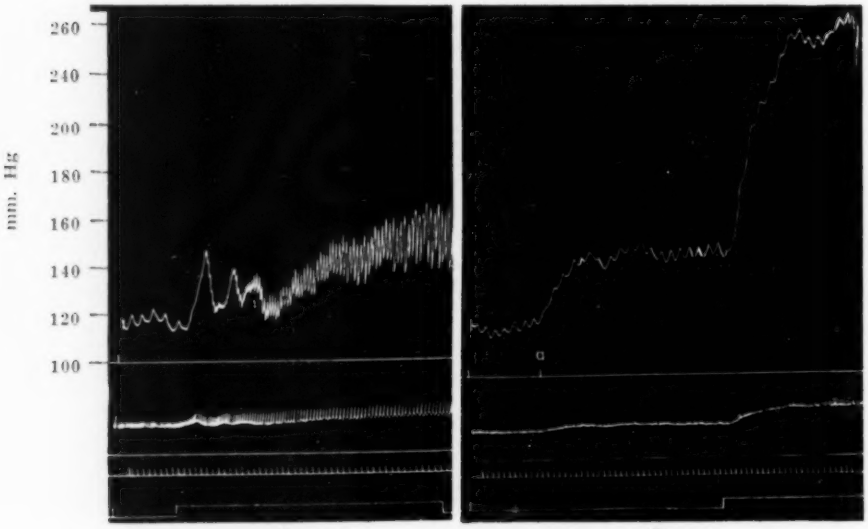

Fig. 5.—Effects of faradizing the peripheral end of the splanchnic nerve. *A*, when both aortic and carotid sinus nerves are intact. *B*, after the two aortic nerves have been severed and the two common carotids have been clamped at *a*. (From Izquierdo, 1930.)

POSSIBLE SIGNIFICANCE OF THESE REACTIONS

Now, let us turn to the question of the relations it might have with the development of hypertension. *Does there exist, in animals or in man, some form of chronic hypertension due to reduced or even absent function of this self-regulating mechanism?*

Experiments in animals have provided a satisfactory answer to this question. Animals cannot live with the high blood pressure produced by the total elimination of the four presso-sensible nerves. But if the nerves of one side are first removed and then those on the other, they survive, especially if important amounts of blood are drawn when the second operation is performed. Rabbits operated upon by Koch and Mies (1929) lived with hypertensions of 150 to 180 mm. Hg (their normal blood pressure being between 90 and 100 mm. Hg) for more than a year and a half, which is about a fourth of their

normal lifetime. I have seen dogs in which high blood pressure (between 200 and 250 mm. Hg) persisted some months after elimination of their two sets of presso-sensible nerves.

But *is hypertension of this kind found in man?* Is there any morbid process capable of suppressing, as in the experiments, all the four presso-sensible nerves? Although it seems that this may happen, at least in certain forms of beriberi polyneuritis (see Koch's monograph), it is not likely that the problem of human chronic hypertension should be referred to as an alteration of this kind.

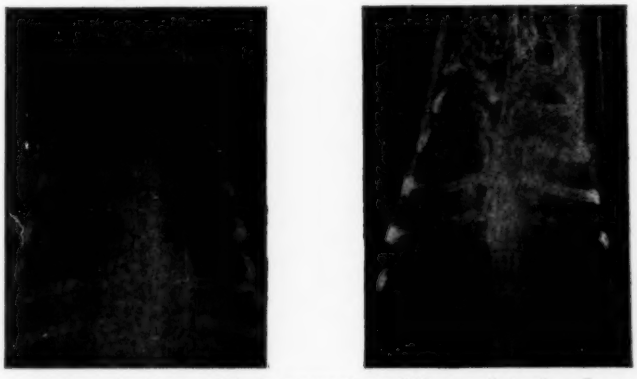

Fig. 6.—Cardiac shadows in the rabbit, in normal condition and twelve days after total suppression of the four presso-sensible nerves. (Plates from Hammer and Mies, 1929)

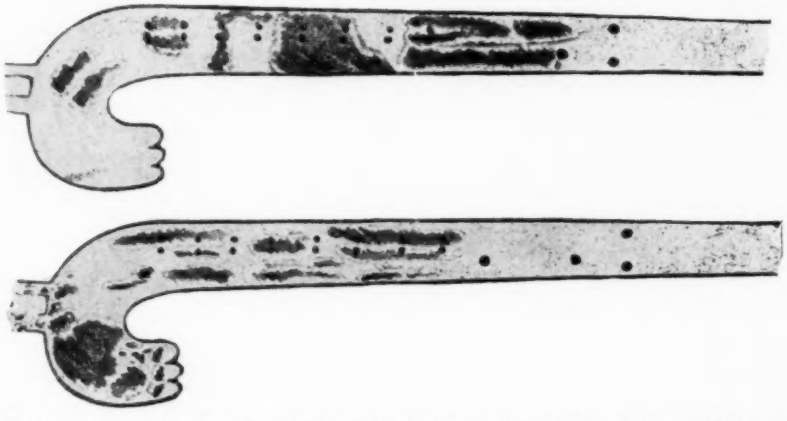

Fig. 7.—Distribution of the sclerotic lesions developed in the aortas of two rabbits after two months of chronic hypertension (140 mm. Hg). (From Nordmann, 1929.)

It is more probable that in man the morbid lesion must be located in the receptors themselves, primarily or secondarily altered by previous changes in the arterial walls, where they are located.

It has been known, for more than twenty years (Hirsch and Thorspecken, 1912), that experimental hypertension, produced and maintained by repeated injections of adrenalin, produces at length calcareous infiltrations and points of lesser resistance in the aortic walls. Furthermore, in animals with chronic hypertension from suppression of all the presso-sensible nerves, radioscopy has shown during life, and postmortem has confirmed after death, that the

heart becomes at first dilated (Fig. 6) and then hypertrophied; that the aorta is also distended and that in this and other arteries the well-known lesions of arteriosclerosis (Fig. 7) are present (Hammer and Mies, 1929; Nordmann, 1929; Mies, 1930). There is no possible doubt about this: hypertension may lead by itself to the production of arteriosclerosis, probably as the result of strain which a continuous exaggerated distention puts upon the circulation within the arterial walls.

According to current views among clinicians this same order of events would take place in man. For them, exaggeration of the arterial tone constitutes the initial trouble, which according to Volhard and his school would be the result of vasoconstrictor substances taking their origin from circulatory and renal deficiencies. But one might suspect, as well, that this might be the result of a primary functional deficiency of the reflex apparatus which regulates blood pressure. This seems to be supported by the fact that in some

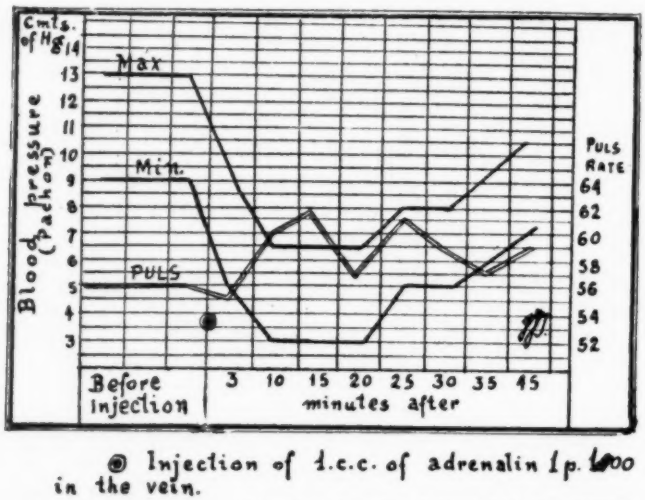

Fig. 8.—Changes in blood pressure and in heart rate, determined by strong reflexes evoked in man by an injection of adrenalin. (From Izquierdo, 1921)

persons hypertension may go for years without leading to the production of cardiovascular lesions. But as is also known, the opposite is true, i. e., some individuals with arteriosclerosis never develop hypertension. It is therefore evident that to solve the problem, additional information is needed upon the functional condition of the units integrating the self-regulating mechanism.

It is desirable to develop some convenient method for testing clinically the functional responsiveness of the whole self-regulating apparatus, to general rises in the general blood pressure, as when adrenalin is injected. To begin with, the normal type of reaction must be found. I have seen that the rises of blood pressure thus produced in persons with highly reactive nerves evoke such powerful reflex actions as to completely counterbalance and even reverse (Fig. 8) the direct pressure-raising action of the drug. Such results are but a mere duplication of those observed in the laboratory. In persons with lowered reactivity of their presso-sensible nerves, the same doses of adrenalin are expected to produce much higher rises of pressure than when

conditions are normal. Of course, such a test will be permissible only in individuals in whom no vascular ruptures might be expected.

The functional reactivity of the two carotid sinuses has been tested by compressing them from outside. The slowing of the heart thus produced was believed, since the investigations of Tschermack, to be due to a mechanical stimulation of the vagus. But Hering proved, first, that this is not stimulated by pressure, and second, that positive results are obtained only when pressure is exerted exactly upon the point where the carotid bifurcates. Compression of

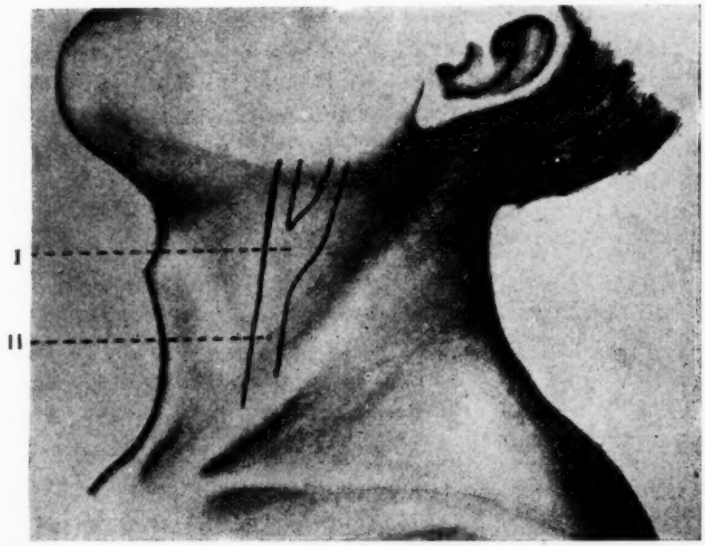

Fig. 9.—The two Hering's points for the carotid compression. (From Hering, 1927)

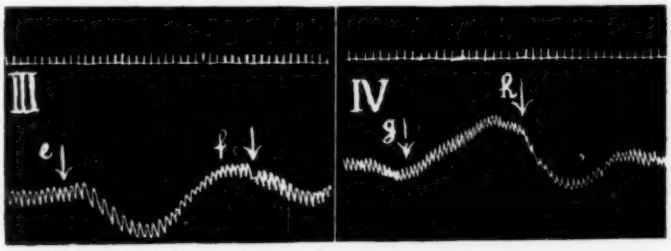

Fig. 10.—The effects evoked in man by compression of the carotid artery. In *III*, the compression is exerted upon the two carotid sinuses between *e* and *f*. In *IV*, both common carotids are compressed from *g* to *h*. (From Regniers, 1930)

point *I* in Fig. 9, where the carotid sinus is felt to beat, evokes in man a double reflex (Fig. 10) which slows the heart and makes the blood pressure fall. Nevertheless, it has been shown that the *starting point for these reflexes is not in the receptors themselves, but in the compressed carotid sinus nerves*, particularly if pressure is more effectively applied upon them through an artery with indurated walls. The method is, therefore, unsuited for getting information on the receptors themselves.

Another method, also developed by Hering, which is but a particular application of the occlusion method formerly referred to, is to be preferred

for this purpose. Pressure is exerted with the tips of three or four fingers upon the middle portion of the common carotid (point *II* in Fig. 9). Thus the carotid sinus becomes more or less emptied of its blood and its receptors cease to be excited by distention of the wall: a double reflex (Fig. 10, *IV*), both of tachycardia and hypertension, ensues. By discontinuing pressure and thus allowing the carotid sinus walls to redistend, with blood rushing in, the two opposite reflexes are elicited (Fig. 10, *IV*). The results are especially marked when both carotids are compressed and decompressed at the same time.

Both Regniers (1930) and Mies (1932) report having obtained very interesting results by using this method. In Regniers' patients with essential hypertension and no cardiovascular lesions, reopening of the carotid never produced either the vagal or the hypertensive reflexes. In the seventeen individuals with essential hypertension observed by Mies, the possibilities of renal lesions were ruled out by submitting them to adequate functional tests. In them, neither carotid compression nor decompression produced cardiac or vasomotor reflexes. In view of all these new data, the existence of a primary and permanent hypertension, due to functional deficiency of the nervous regulatory apparatus, seems to be on its way toward recognition.

Observations have also been reported which show that sclerosis of the arterial walls at the points where the receptors lie, may lead to elimination of the self-regulating mechanism (Hering, 1927). This would either create hypertension, or if hypertension is already present, convert it into an irreparable process.

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COLIMA NUM. 367

AN ABRIDGED KEY TO THE SPECIES OF PATHOGENIC FUNGI*

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THIS key is a companion to "An Abridged Key to the Genera of Pathogenic Fungi," which appeared in this JOURNAL.† A synopsis for most of the pathogenic species of the genus is given. It must be borne in mind that these organisms vary in their morphology on different culture mediums, and that they may vary on the same sort of medium.

In the instances of the species of the saprophytes, the more common members are keyed, but summary is given.

Detailed literature should be consulted for the study of the Dermatophytes.

The Genera in this key are numbered as in the *key to the genera.*

1. KEY TO THE SPECIES OF THE GENUS EPIDERMOPHYTON

The better known species of this genus may be recognized as follows:

Lemon yellow growth on Sabouraud's agar

1. *E. cruris*

Pale rose

E. perneti

Deep red

E. rubrum

The less known species which have been described are:

E. interdigitale, E. lanoroseum, E. nivenum, E. plurizoniforme, and E. salmoneum.

1. *Epidermophyton cruris* Castellani, 1905.

Examination of the skin in hydroxide shows an abundance of filaments 4-5μ in diameter, composed of quadrangular cells joined end to end and dissociating readily. These filaments are developed horizontally in hardened areas of the epidermis. The deeper layers of the skin are not invaded.

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Received for publication, May 2, 1935.
†Shaw, Frederick W.: J. LAB. & CLIN. MED. **20**: 113, 1934.

On culture mediums, growth is slow. The color is lemon yellow, dry and powdery, but becomes rapidly downy and white, by pleomorphism. Microscopic examination reveals the characteristic pluriseptated spindles.

Pathogenesis: Eczema marginatum or Tinea cruris.

2. KEY TO THE SPECIES OF THE GENUS TRICHOPHYTON

The more common species may be classified as follows:

Endothrix

Cultures acuminate

Growth white

1. *T. acuminatum*

Growth violet

2. *T. violaceum*

Cultures crateriform

Yellow center with white periphery

3. *T. crateriforme*

Cultures crateriform and creased; white

4. *T. plicatile*

Cultures cerebriform

5. *T. cerebriforme*

Ectothrix

Small spores (3μ); growth rapid

Growth starlike, powdery surface, plaster-like; frequently coiled bodies

Gypseum group

Growth snowflake-like, downy; coils and spindle spores lacking

Nivenum group

Large spores (5-8μ)

Megaspore group

1. *Trichophyton acuminatum* Blanchard, 1895.

When the hair is examined in hydroxide, short and very fragile mycelial elements, which dissociate into round, oval or cylindrical spores of from 5 to 7μ in diameter, are seen.

On culture mediums the growth begins in the form of a small hemispherical cone, surmounted with three or four fimbriated, pinnate points. Older cultures may become lacunar.

Pathogenesis: Ringworm of hair, skin, nails.

2. *Trichophyton violaceum* Bodin, 1902.

This endothrix fungus was discovered by Sabouraud in cases of dry tinea of the scalp and beard. On culture it grows with the form of a slight disk, acuminated at the center. The growth is thin, moist, and pale brown or gamboge in color at the beginning; later it becomes larger. Small riddled furrows are formed, and in about three weeks the culture becomes a violet color. This color is lost after a number of transplants. No spores are formed in cultures.

3. *Trichophyton crateriforme* Sabouraud, 1902.

When examined in hydroxide, the hair is seen to be filled with simple filaments, formed by a series of quadrangular spores of 4 to 5μ in length, giving the appearance of a ladder. Growth on maltose medium begins as a white growth, which extends until the border is raised. The consistency of the colony is velvety, at first, later becoming dry and powdery. As growth continues, the color becomes creamy.

Pathogenesis: Ringworm of the scalp, herpes circinata of the smooth skin, ringworm of the nails, and dry trichophytosis of the eyelashes.

4. *Trichophyton plicatile* Sabouraud, 1909.

This fungus may be found around the hair. Cultures resemble those of *T. crateriforme*. They are white and powdery, with their surfaces folded at the center; the periphery presents a powdery zone.

Pathogenesis: Dry and wet trichophytosis of the beard.

5. *Trichophyton cerebriforme* Sabouraud, 1893, 1909.

The skin, when examined in hydroxide, resembles that of *T. crateriforme*. On culture this fungus may be distinguished from *T. crateriforme* by the chiffon appearance of its surface and its color, which becomes cream in old cultures. The periphery of the colony shows powdery-like rays, distinct and unequal, while that of *T. crateriforme* is a powdery-like aureole.

Pathogenesis: Herpes circinata, ringworm of the scalp and beard.

3. KEY TO THE SPECIES OF THE GENUS ACHORION

Yellowish colonies, slight duvet in the center, the remainder smooth
1. *A. gypseum*

Colonies uniformly white with duvet
2. *A. quinckeanum*

Colonies light yellow, cerebriform
3. *A. schoenleini*

1. *Achorion gypseum* Bodin, 1907.

Colony flat, yellowish, with a little tuft of white down in the center and a white border. Isolated from a case of favus.

2. *Achorion quinckeanum* Zopf, 1890.

In the lesions the mycelial filaments are abundant and are broken up into small segments which constitute the spores. On culture medium the growth is uniformly white with a duvet, and a wrinkled center. The organs of reproduction appear about the fourth day and are of the Acladium type. The cause of Mouse Favus; transmissible to man.

3. *Achorion schoenleini* Remak, 1845.

The fungus may be demonstrated in either the scutulum or hair, and appears as rounded cells, oval cells, and short and long filaments. Colonies are at first yellowish and waxy, becoming much wrinkled with age. Some strains develop whitish aerial hyphae. This fungus is extremely pleomorphic. The cause of favus.

4. KEY TO THE SPECIES OF THE GENUS MICROSPORON

Radial furrows; small central, elevated knob.
1. *M. audouini*

No furrows
Central portion of colony flat
2. *M. lanosum*
Small central knob of light yellow
3. *M. felineum*

1. *Microsporon audouini* Gruby, 1843.

Clarified with hydroxide or other fluids, the hair presents, under the microscope, a multitude of small spores 2 to 3μ in diameter, polyhedral and doubly contoured. Hyphae on inside, spores on outside of hairs. The colonies are snow white with a small central knob elevated above the surface, and containing from four to six deep clefts. The whole surface of the colony is covered with a fine duvet. Cultures grow slowly. Causes ringworm of scalp.

2. *Microsporon lanosum* Sabouraud, 1907.

Hairs in hydroxide similar to *M. audouini*. Colonies show a central flat elevated ring of aerial hyphae; no folding; woolly surface; become reddish in the center, when spindle spores are formed. Causes ringworm of the scalp.

3. *Microsporon felineum* Fox and Blaxall, 1896.

Rapid growth on culture medium. Colony regular in form, with a small central disk of a yellowish color. The periphery is composed of a fine radiating duvet.

Pathogenesis: Tinea capitis very often accompanied by cutaneous lesions.

5. KEY TO THE SPECIES OF THE GENUS ENDODERMOPHYTON

Cultures become black
Rapidly
1. *E. mansoni*
Slowly
2. *E. concentricum*
Cultures red
3. *E. indicum*
Cultures light yellow
4. *E. tropicale*

1. *Endodermophyton mansoni* Castellani, 1914.

Direct examination of the scales of the skin in hydroxide shows a network of mycelial filaments of a greenish yellow color, and are composed of oval or rectangular elements of unequal dimensions, or of chains of spores. Slow growing; at first amber in color, later black; no duvet. Found in *Tinea imbricata*.

2. *Endodermophyton concentricum* R. Blanchard, 1895.

Differs from the above by its cultures which slowly turn black. Found in the same conditions.

3. *Endodermophyton indicum* Castellani, 1911.

Differs from the above by its yellow cultures and duvet. Later the growth becomes red.

4. *Endodermophyton tropicale* Castellani, 1914.

Examination of the scales of the skin similar to the above. Colonies crinkled, slight amber color, becoming deeper with age; duvet usually absent. Found in the same condition as the above species.

6. KEY TO THE SPECIES OF THE GENUS MALASSEZIA

Causes a brown eruption on white skin
1. *M. furfur*

Causes a reddish yellow eruption on white skin
2. *M. tropica*

1. *Malassezia furfur* Ch. Robin, 1852.

When the skin scrapings are examined in hydroxide solution, the fungus is seen in abundance either in the form of filaments or of rounded spores. The filaments measure about 3μ in diameter, are unbranched, flexuous, continuous or septate. The spores are from 3 to 5μ in diameter, generally in masses of from 15 to 30 elements. Said to have been cultivated on glycerinated gelatin. Causes pityriasis versicolor in man.

2. *Malassezia tropica* Castellani, 1908.

In the epidermis this fungus produces a thick mycelium with irregular contractions. Causes a reddish yellow eruption on the white skin and a light yellow eruption on the dark skin. Causes Tinea flava.

7. KEY TO THE SPECIES OF THE GENUS TRICHOSPORUM

Nodules on hair polyhedral
3-4μ in diameter
1. *T. beigeli*
5-8μ in diameter
2. *T. hortai*
12-14μ in diameter
3. *T. giganteum*

1. *Trichosporum beigeli* Rabenhorst, 1867.

On the hair this fungus produces irregular nodes which, under the microscope, are seen to be made up of elements about 3 to 4μ in length. In culture, rounded cells of about 3μ are obtained, which elongate into septated and branched filaments. Chlamydospores are present in old cultures. These are thick and refractive, isolated, serrated, intercalated or terminal. They may be as large as 12μ in diameter. Causes Trichosporosis of the beard.

2. *Trichosporum hortai* Brumpt, 1913.

The nodules on the hair are polyhedral and are from 5 to 8μ in diameter. Growth on culture medium is slow; greenish in color, later brownish black. Strongly attached to the substratum. Grows on potato. Causes Piedra.

3. *Trichosporum giganteum* Behrend, 1890.

Direct examination of the hair shows polygonal cells from 12 to 14μ in diameter, forming the nodes. Except at the epidermal margin, the hair is not much altered by the invading fungus. Examination of the cultures re-

veals filaments from 10 to 60μ in length, by 1 to 4μ in width, frequently branched, septate. The spores are of variable size and shape, in masses or in chains, 2 to 12μ in diameter. Causes Piedra.

8. KEY TO THE SPECIES OF THE GENUS INDIELLA

Grains hard and beanshaped
1. *I. mansoni*

Grains soft, composed of mycelium in coiled masses
2. *I. reynieri*

1. *Indiella mansoni* Brumpt, 1906.

This fungus is known only in the parasitic stage. The mycelium is white, delicate when young, and measures about 1 to 2μ, but some elements may be 15 to 20μ. The old filaments become irregular, 3 to 5μ in diameter; the septa vary from 5 to 10μ. Chlamydospores are generally terminal, rarely intercalated, of from 5 to 12μ in diameter, generally spherical, unicellular, at times segmented. The granules are from $\frac{1}{5}$ to $\frac{1}{4}$ mm., flat. Causes mycetoma.

2. *Indiella reynieri* Brumpt, 1906.

Mycelium white, living as a parasite in man. The young mycelium is delicate, of a diameter of 1 to 5μ; the septa are from 10 to 15μ in length. The peripheral filaments are irregular, moniliform, acquire a diameter of from 4 to 5μ. Most of the filaments present chlamydospores at the ends, which are often divided into two or three pieces. The filaments always remain rolled up or twisted, and form a characteristic granule, which is round when young, but twisted and cordlike when old. The granules do not exceed 1 mm. in diameter. This organism causes white grain mycetoma.

9. KEY TO THE SPECIES OF THE GENUS MADURELLA

Numerous black sclerotia at periphery of cultures
1. *M. americana*

Sclerotia formed below the surface of culture medium
2. *M. mycetomi*

Sclerotia rarely produced; when present, on surface only
3. *M. tozeuri*

1. *Madurella americana* Gammel, Miskdjian, and Thather, 1926.

Black granules of about 1 mm. in diameter in masses of from 3 to 5 mm. Structures resembling chlamydospores may be seen after treatment with hydroxide. On culture the growth is at first white, then becomes more and more brown, which is followed by a diffusion of the pigment into the medium. This pigment is secreted from numerous sclerotia at the periphery of the culture. The cause of American mycetoma.

2. *Madurella mycetomi* Laveran, 1902.

Black granules, small, hard, rounded, verrucose. Growth on mediums grayish white, becoming yellowish, then blackening the medium. Oidia of from 2 to 5μ. Sclerotia sterile, black, ½ to 1 mm. The cause of mycetoma with black granules.

3. *Madurella tozeuri* Nicolle and Pinoy, 1908.

Black granules of amorphous structure, the granules being composed, often, of a mycelial ring enclosing degenerated cellular elements. The pigment is derived from the fungus in these masses. The growth on culture medium is at first white, becoming yellowish with age, followed by blackening of the medium. Causes mycetoma with black granules.

10. KEY TO THE SPECIES OF THE GENUS BLASTOMYCOIDES

In tissues with a thick mucous capsule
1. *B. histolytica*
Thick mucous capsule not present in tissues
Reproduce by budding
2. *B. dermatitidis*
Reproduce by endospores
3. *B. immitis*

1. *Blastomycoides histolytica* Stoddard and Cutler, 1916.

This organism appears in the tissues as round, budding cells, with a thick capsule of mucoid material. In cultures no capsules are present, no mycelium is formed, no ascospores have been observed. Growth is slow, creamy; most abundant on potato and dextrose agar. No gas is formed from carbohydrates. The cause of infection of the brain and meninges.

2. *Blastomycoides dermatitidis* Gilchrist and Stokes, 1898.

In the tissues the fungus occurs as a round or oval budding cell, with a thick and highly refractile wall. No endospores are present. Growth on culture medium may present three types and transitions between them. The one may resemble the growth of the tubercle bacillus, another the acladium and the third the cottony type. Yeastlike cells and branching, segmented hyphae may be seen on microscopic examination. This will vary with the age of the culture and other factors. This organism causes blastomycosis.

3. *Blastomycoides immitis* Bixford and Gilchrist, 1897.

In the lesions, roundish bodies which vary from 3 to 80μ in diameter; generally with a thick capsule (although the small forms may have a thin capsule) which is well defined. Within some of these structures are numerous spores. Reproduction by budding never occurs. On culture medium the fungus may be of two types: a yeastlike type, reproducing by budding, and a filamentous type. The colonies are round and somewhat elevated. Old cultures may show chlamydospores. The mycelium is septate and branched. An abundance of white aerial hyphae is formed; no conidia are present. The growth resembles that of *B. dermatitidis*, but differs microscopically in the production of large numbers of chlamydospores. This fungus produces coccidioidal granuloma.

11. KEY TO THE SPECIES OF THE GENUS HEMISPORA

Colonies asteroid
1. *H. stellata*
Colonies cerebriform
2. *H. rugosa*

1. *Hemispora stellata* Vuillemin, 1906.

In the lesions, direct examination reveals the mycelium in the form of white disks, ½ to 2½ mm. in diameter, sessile, covered with conidiophores designing stars on the surface. In culture, irregular growths are obtained with a stellate appearance. Isolated from osseous lesions and cold abscesses.

2. *Hemispora rugosa* Castellani, 1910.

This species produces cerebriform colonies. Isolated from sputum.

12. KEY TO THE SPECIES OF THE GENUS DEMATIUM

Conidia globose, spiny, in chains
D. atro-purpureum

Conidia pear-shaped, warty, in chains
D. scabridum

Conidia globose, smooth, short chains
D. castaneae

13. KEY TO THE SPECIES OF THE GENUS HAPLOGRAPHIUM

Conidia apiculate
H. apiculatum

Conidia elongated, elliptic
H. atro-brunneum

14. KEY TO THE SPECIES OF THE GENUS CLADOSPORIUM

Pathogenic

Associated with Tinea nigra
1. *C. mansoni*

Associated with ulcerating nodules
2. *C. penicillioides*

Saprophytes

Hyphae pale green
C. epiphyllum

Hyphae brown or olive
C. herbarum

1. *Cladosporium mansoni* Castellani, 1908.

In the skin the fungus appears as elongated filaments of from 18 to 20μ in length, by 2 to 3μ wide; septate, unbranched. The rounded spores are 5 to 10μ, and are often in masses. Black pigment is contained in all of these structures. On culture medium, colonies appear in from two to four days; black, becoming confluent, adherent to the substratum. Causes Tinea nigra.

2. *Cladosporium penicillioides* Preuss, 1848.

In the tissues the fungus appears in the form of ovoid or fusiform masses, 3 to 4μ in length. Cultures are readily obtained. These are chocolate brown, with a powdery surface; cerebriform colonies. This organism was isolated from an ulcerated nodular lesion of the leg.

15. THE GENUS MONILIA

For the classification of the species of this genus, the reader should consult special articles on this subject.

16. THE GENUS ENDOMYCES

The following species have been described, but the available data are insufficient for differentiation: crateriforme, cruzi, molardi, pulmonalis and vuillemini.

17. KEY TO THE SPECIES OF THE GENUS RHIZOMUCOR

Columella piriform

1. *R. parasiticus*

Columella spherical

2. *R. septatus*

1. *Rhizomucor parasiticus* Lucet and Costantin, 1900.

Colonies grayish to yellowish brown. Sporangiophores 1 to 2 mm. in length, ramified, rarely branched a second time, generally in clusters; sporangia 35 to 80μ with a membrane bordered with fine crystalline needles; ovoid, piriform, hardened, slightly brownish columella 30 to 70μ in height by 25 to 55μ in width; lateral sporangia smaller. Spores round or oval, 4 by 2.5μ. Zygospores absent. Isolated from sputum in pseudotuberculosis.

2. *Rhizomucor septatus* von Bezold, 1889.

Sporangiophores brownish, ramified, in clusters, average diameter 10μ; small rhizoids at the base; secondary sporangiophores 3 to 4μ, short with transverse septa at the point of ramification. Sporangia pale grayish brown, spherical, with a transparent membrane, 32μ in diameter. Columella spherical or slightly ovoid, brown, 25μ in diameter. Spores spherical or oval, thin, yellow or brownish. This organism has not been cultivated. Isolated from the external auditory canal.

18. THE GENUS RHIZOPUS

Rhizopus niger Ciaglinski and Hewelke, 1893.

Stolons branched, with numerous rhizoids, forms a snow white mat. Upright sporangiophored pedicles, straight, fasciculated, terminated by spherical sporangium black at maturity. Columella at first cylindrical, 2 to 3 times longer than their width, enlarging later and presenting at maturity the appearance of a spherical cap; caving in after drying out of the sporangium and taking the form of an open umbrella. Spores oval, thin, gray. Zygospores heterothallic. Recovered from black tongue.

19. THE GENUS ABSIDIA

This genus contains no pathogens, at this time.

20. THE GENUS MUCOR

For the identification of species of this genus the reader should consult special monographs.

21. KEY TO THE SPECIES OF THE GENUS FUSARIUM

Conidia brownish to salmon color
Conidiophores simple or slightly branched
F. orthocerus
Conidiophores much branched; macroconidia not broader toward apex
Bluish-black sclerotia on potato
F. oxysporum
Sclerotia absent
F. lini
Conidia brown-white to golden brown, walls thick
F. solani
Conidia cream-buff to cinnamon
F. caudatum

22. KEY TO THE SPECIES OF THE GENUS ALTERNARIA

Conidia smooth
Colonies brown-green
1. *A. tenuis*
Colonies brown
A. fasciculata
Conidia rough
A. humicula

1. *Alternaria tenuis* Ness, 1817.

Colonies brown. Conidiophores short, septate, branched or unbranched, brown green. Conidia in chains, with cross and longitudinal walls (3 to 5 cross walls), constricted at the outer walls, olive green or brownish black, variable in size and shape, 30 to 35μ by 15μ. Usually saprophytic, but has been reported as pathogenic.

23. THE GENUS ACROTHECA

Acrotheca pedrosoi Fonseca and Leao, 1923.

This is probably a blastomycoides. In the tissues are found ovoid or spherical blackish corpuscles within the giant cells. On culture, well-developed hyphae, 2.5 to 3.2μ in diameter, with septa 17 to 24μ in length; blastospores 5 to 8μ by 3.5μ; conidia 1.5 by 4.5μ. Causes a verrucose dermatitis.

24. THE GENUS PHIALOPHORA

Phialophora verrucosa Thaxter, 1915.

This organism is probably a blastomycoides. Occurs in the tissues in two forms: sclerotes and budding forms. The sclerotes are at first unicellular and vary from 8 to 15μ in diameter; their walls are colored blackish brown. Septation soon follows with the production of sclerotes, pleuricellular, and budding forms which in turn may be transformed into sclerotes. Cultures are black, with a water soluble pigment. Branches of aerial hyphae end by sporogenous cells in the form of hollow cupola, which produce conidia 4 to 6μ by 2μ, and which remain agglomerated in masses of 4 to 20 elements, in the

form of globular false sponges, at first yellowish, then brown. Sclerotes, similar to those in the tissues, are formed in ascitic gelatin and coagulated serum, and are terminal, septate. Conidial buds are produced. Causes a verrucose dermatitis.

25. KEY TO THE SPECIES OF THE GENUS ACREMONIELLA

Pathogenic
1. *A. perini*
Saprophytic
Conidia smooth
Pale ochre, finally olive
A. fusca minor
Black
A. melanosperma
Conidia rough, dark brown
A. brevia

1. *Acremoniella perini* Pollacci, 1923.

Characterized by short conidiophores which give rise to a single brown conidium, finely echinulated. Isolated from sputum.

26. KEY TO THE SPECIES OF THE GENUS CEPHALOSPORIUM

Conidiophores branched; growth pure white
C. curtipes
Conidiophores unbranched; growth white, then rose
C. acremonium

C. acremonium has been reported as being pathogenic.

27. KEY TO THE SPECIES OF THE GENUS TRICHODERMA

Colonies white
T. album
Colonies green
Conidia globose
T. lignorum
Conidia ovoid to elliptic
T. glaucum

28. KEY TO THE SPECIES OF THE GENUS ACREMONIUM

Colonies finally orange
1. *A. potroni*
Colonies generally white
2. *A. niveum*
Colonies finally red
3. *A. muthuoni*

1. *Acremonium potroni* Vuillemin, 1911.

Hyphae septate; conidiophores numerous, elongated, from 15 to 20μ long; conidia ovoid, smooth, 4 to 5μ long, 2μ wide, pinkish; colonies at first white, then pink to orange. Isolated from papules.

2. *Acremonium niveum* Boucher, 1918.
Similar to *A. potroni*, colonies are generally white.
3. *Acremonium muthuoni* Fontoynont and Boucher, 1922.
Similar to *A. potroni*, but colonies become brick red.

29. THE GENUS CEPHALOTHECIUM

This genus is of no pathogenic importance.

30. THE GENUS DIPLOSPORIUM

Diplosporium vaginae Nannizzi, 1925.

Sporophore branched with a terminal conidia that is hyaline, oblong, bicellular, smooth, 15-20μ by 5-6μ. When the conidia are detached, they are replaced by another growing one, but remain joined together for a long time by means of a gelatinous membrane. Chlamydospores and isolated or clustered aleuriospores present. Isolated from a case of purulent vaginitis.

31. THE GENUS ALLANTOSPORA

Allantospora onychophila has been reported as being pathogenic, but there is doubt as to the classification of this organism.

32. KEY TO THE SPECIES OF THE GENUS CITROMYCES

Wehmer has described two species of this genus which produce citric acid from sugar. Thom places them with the Penicillia.

Conidia smooth
C. glaber
Conidia bristly
C. pfefferianus

33. KEY TO THE SPECIES OF THE GENUS ASPERGILLUS

This is a large genus. The monograph by Thom and Church should be consulted for detailed information. The more important species are given below.

Conidia white
A. candidus
Conidia colored
Green, bluish green, yellowish green or gray
Perithica readily produced
Perithicum embedded
Sterigmata apical
A. fumigatus
Sterigmata pleurogenous
A. clavatus
Perithicum not embedded
A. glaucus
Perithica not readily produced, if at all;
conidiophore rough

A. flavus-orzzae

Blackish brown

A. calyptratus-luchuensis

Light brown

A. wentii

A few species of the genus Aspergillus cause infection in man.

34. KEY TO THE SPECIES OF THE GENUS STERIGMATOCYSTIS

Conidial forms blackish brown

S. nigra

Conidial forms green, then gray to brown

S. nidulans

The two species have been reported as pathogenic.

35. KEY TO THE SPECIES OF THE GENUS PENICILLIUM

Colonies white

P. candidum

Colonies white, later yellow

P. wortmanni

Colonies yellow or brown

P. brevicaule

Colonies red

P. roseum

Colonies green

Glaucum, italicum, olevaceum, granulatum, claviforme, areum, purpurogenum, luteum

Consult "The Penicillia," by Thom, for details of this genus.

36. KEY TO THE SPECIES OF THE GENUS ACLADIUM

Pathogenic

1. *A. castellanii*

Saprophytic

A. densissimum

1. *Acladium castellanii* Pinoy, 1916.

Colonies consist of roundish masses, which may coalesce; covered with spicules, which give the colony a prickly appearance; pseudoconidia of variable shape, cylindriform, pyriform or spherical, lateral, attenuated in size at their points of insertion, 4 by 3μ. Colonies on carrot and potato are white, on dextrose agar often amber; old cultures brown or black.

37. THE GENUS ALEURISMA

The following species have been reported, but in most instances the data are insufficient for differentiation: *A. arloingi* (probably an achorion), *A. benignum*, *A. lugdunense* and *A. salmoneum*.

38. THE GENUS CORETHROPSIS

But one species has been reported from *C. hominis*. This fungus is known only in the parasitic state. It was found in a trichophid lesion of the forearm.

39. KEY TO THE SPECIES OF THE GENUS GLENOSPORA

Aleuriospores 6 to 10μ by 5 to 8μ

1. *G. grandavensis*

Aleuriospores 5 by 3μ

2. *G. graphii*

1. *Glenospora grandavensis* Vuillemin, 1921.

Cultures blackish, covered with a white dust, formed from dissociated colorless pieces. The aleuriospores are 6 to 10μ by 5 to 8μ. Neither conidia nor endoconidia are present. Isolated from fetid bronchitis sputum.

2. *Glenospora graphii* Vuillemin, 1912.

Colonies at first colorless, later become brownish. The filaments are of the same color, branched, septate, 2 to 3μ in diameter. Aleuriospores are ovoid, thin walled, 5 by 3μ, become brown at maturity. These spores are isolated at the extremity of the branches. The straight filaments can effect a fasciculated appearance. The branches are arranged totally without order, nearly irregularly alternating and then subopposed or subverticulated. Causes otomycosis and keratomycosis.

40. KEY TO THE SPECIES OF THE GENUS SPOROTRICHUM

There is probably but one species pathogenic for man

1. *S. schenckii*

Nonpathogenic

Colonies red

S. roseum

Colonies white, cottony

S. pruinosum

1. *Sporotrichum schenckii* Hektoen and Perkins, 1900.

In the tissues and pus the fungus appears as small (3 to 4μ by 1.5 to 3μ) spindle-shaped cells. In the pus treated with hydroxide these cells appear as highly refractile spores. They are gram-positive and generally occur within the polymorphonuclear leucocytes. No mycelium develops in the body.

Cultures present at first a soft, creamy, moist, shiny growth. As the culture ages, it becomes a light tan, deepening to a brown or black in most strains. The surface is wrinkled.

Microscopic examination of the growth from dextrose agar shows the organism to consist of branching hyphae from which ovoid conidia develop either from the sides of the hyphae or from the lateral or terminal filaments. Causes sporotrichosis.

41. THE GENUS MYCODERMA

This genus is of no pathogenic importance.

42. THE GENUS TORULA

There are no species of interest to the pathologist in this genus.

43. THE GENUS CRYPTOCOCCUS

Cryptococcus hominis Vuillemin, 1901.

In the pus, oval bodies with a double contour. The elements are arranged in groups, each group embedded in an amorphous substance, surrounded with a capsule. Growth on culture medium white or slightly yellow. Yeastlike forms from culture; no mycelium; no asci. Found in abscesses.

44. THE GENUS DEBARYOMYCES

Probably the most important pathogenic species of this genus is:

Debaryomyces hudeloi (de Beurmann and Gougerot, 1909), Fonseca emen. 1922.

Cells round, oval or elongated; 2 to 4μ in diameter, by 6 to 8μ long. Asci scanty, one spore, verrucose surface. Isolated from multiple abscesses.

45. THE GENUS SACCHAROMYCES

Monographs on the yeasts should be consulted for the species of this genus.

46. THE GENUS WILLIA

Willia anomala Hansen, 1904.

Cells oval, small; asci contain two or four spores of the hatlike type; dextrose and beer-wort fermented, but not maltose or saccharose; a membrane is formed in sugar liquid mediums (the membrane contains gas bubbles). Ethers are produced from sugar mediums. Isolated from sputum.

FERMENTATION AND GAS PRODUCTION BY B. COLI IN SIMPLE AND MIXED SUGARS*

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INTRODUCTION

THE choice of carbohydrate for modification of cow's milk in infant feeding has concerned pediatricians during the past several decades. Some clinicians have expressed themselves as being unable to detect significant differences among the various sugars. Yet one would expect, a priori, that some differences must exist even though so slight as to be overlooked in isolated or limited clinical observation. Other clinicians have definite preferences for various carbohydrates based either upon indefinite clinical impressions of superiority or upon the idea that certain carbohydrates are less readily attacked by the bacteria of the intestinal flora and thus cause less fermentation, gas and distention than do other sugars.

*From the Department of Preventive Medicine, New York University College of Medicine.

Received for publication, May 3, 1935.

This investigation was made possible by a grant from The Burroughs Wellcome and Company (U. S. A.) Experimental Research Laboratories to whom we wish to express our thanks.

The object of this study was to test this hypothesis by experiments in vitro. It seems reasonable to assume that single representatives of the intestinal flora will show differences in the fermentation of various carbohydrates in vitro similar to the differences which they show in the intestine. Although it is recognized that strict conclusions concerning in vivo processes cannot be drawn from in vitro experiments, such experiments are useful inasmuch as they may point to certain possibilities concerning in vivo processes.

Since *B. coli* is the main representative of the intestinal flora, strains of this organism were studied. They were obtained from the stools of two groups of infants, the subjects of a clinical comparison of cane sugar with a high dextrin carbohydrate.

The studies of Rettger and Cheplin,[1] Cruickshank,[2] Nissle,[3] and von Knorr[4] are of interest in relation to this work.

EXPERIMENTAL METHODS

The infants were fed a standard formula including milk and either cane sugar or carbohydrate C (high dextrin) as the sole additional sugar. The stools were tested at intervals of two weeks. Strains of *B. coli* were fished and tested separately.

The isolated strains were cultured in fermentation tubes. The basic medium consisted of 0.5 per cent peptone (Difco) solution adjusted to pH 6.9-7.1. The carbohydrate was made up as a 10 per cent solution in 0.5 per cent peptone, sterilized by filtration through a Berkefeld, tested for sterility, and kept in the ice box. To prepare the media for the studies, sufficient of the 10 per cent carbohydrate solution was added (with aseptic precautions) to a definite quantity of sterile 0.5 per cent peptone solution to give the desired 0.5 per cent concentration of carbohydrate. Sterile Smith fermentation tubes were filled with 50 c.c. of sterile 0.5 per cent carbohydrate medium and incubated twenty-four hours for sterility.

Cultures of the *B. coli* were grown for eighteen hours on extract agar slants. The growth was washed with 2 c.c. of saline, and 0.1 c.c. of this suspension of bacteria inoculated into the Smith fermentation tubes containing the various carbohydrates. The tubes were incubated for twenty-four hours at 37° C. Smears were made from each tube and stained with gram stain to check the purity of the cultures.

The carbohydrate materials used in these fermentation studies included glucose, maltose, lactose, and carbohydrates A, B, and C. The last three are commercial preparations, A being in the nature of a syrup and B and C prepared from starch by partial hydrolysis. The chemical tests carried out before and after the bacterial growth included the amount of gas, pH, total acidity, and reducing actions both total and unfermentable.

The determinations were carried out as follows:

Gas.—The length of the gas column in the Smith tube was measured with a ruler with divisions in millimeters. The results are given as millimeters, and while perhaps not as accurate as might be wished for, are sufficiently so for the purposes in view.

pH.—Indicators and standard controls were used.

Total Acidity.—To 10 c.c. of medium (before and after growth of organisms) in a 50 c.c. Erlenmeyer flask were added about 10 c.c. of distilled water and between 1.00 and 5.00 c.c. of accurately measured 0.1 N sulphuric or hydrochloric acid. The mixture was boiled for one to two minutes, cooled, and titrated with 0.1 N sodium hydroxide solution with phenolphthalein as indicator. The results are given as cubic centimeters of 0.1 N sodium hydroxide used, or tenths of milli-equivalents of acid present in 10 c.c. of medium.

Reducing Actions.—Total, unfermentable, and fermentable (by difference). The 1926 modification of Benedict's method was used.[5]

1. Preparation of filtrates: To 4 c.c. of medium were added 4 c.c. of distilled water, or (for unfermentable reductions) 4 c.c. of yeast suspension (i.e., one cake of Fleischmann's yeast suspended in 100 c.c. distilled water). The yeast fermentation was warmed somewhat (40° C.) and allowed to stand fifteen minutes. Then 1 c.c. of 10 per cent sodium tungstate solution and 1 c.c. of 0.7 N sulphuric acid were added and the mixture shaken, using a rubber stopper. After thirty minutes, the 10 c.c. mixture was filtered through 11 cm. Swedish paper.

2. Dilution and analysis: Because 2 c.c. of the filtrates of the different carbohydrate media were too strongly reducing to read against the glucose standard (0.2 mg. glucose in 2 c.c.), the following dilutions were used: glucose 2:25; maltose 1:8; lactose 1:8; A 1:4; B 1:4; C 1:4. To 2 c.c. of the diluted filtrate in a Folin-Wu sugar tube were added 2 c.c. of the alkaline copper reagent, the mixture shaken, heated in boiling water for five minutes, cooled under running water, 2 c.c. of the complex tungstic acid color reagent added, allowed to stand two to three minutes, diluted to the 25 c.c. mark, shaken, and read in the colorimeter against the standard (0.2 mg. to 2 c.c.) glucose solution set at 30 mm.

$$\text{Calculation.—Mg. (calc. as glucose) per c.c. medium} = \frac{0.2 \times 30}{\text{Reading in mm.}} \times \tfrac{1}{2} \times \text{Dilution} \times$$

$$\frac{10}{4} = \frac{15}{2} \times \frac{\text{Dilution}}{\text{Reading}}$$

EXPERIMENTAL RESULTS

In every experiment with *B. coli* in the different carbohydrate media, the medium without the culture was analyzed as well as after incubation with the culture. Because of the method of preparing the media, especially the high concentrations of the carbohydrate solutions added, there was a certain variation in the chemical compositions of the individual media, although these differences were of small significance in the study of the utilization of the constituents by the *B. coli*.

The analyses of the media are given in Table I. The individual determinations are not included. Only the extreme values, the averages, and the number of determinations are given.

The pH determinations ranged from 6.8 to 7.3, values which are within the limits of accuracy used in the preparation and testing of the media. The averages of the titrable acidities of the different carbohydrate media were practically the same, 0.28 to 0.31, although individual values ranged between

0.1 and 0.6. The glucose media were made up to contain 0.5 per cent glucose. The reducing actions gave an average value within 10 per cent of this, while the largest individual difference was 33 per cent. Ordinary pipettes, etc., were used so that some variation in individual measurements occurred. All of the glucose was fermentable, while with the maltose and lactose media no yeast-fermentable material was found by the tests as described, i.e., no glucose was present. In the carbohydrate A (a syrup) medium, about one-third of the reducing action was due to glucose. The nature of the materials causing the remaining reduction was not determined. Carbohydrate B medium contained 0.035 per cent glucose, while C contained practically none. Assuming maltose to be the only other reducing substance present, from the results of the maltose media, B medium contained an average of 0.23 per cent maltose, and C medium an average of 0.18 per cent maltose. These results, especially the glucose values, indicate that carbohydrate B was broken down or hydrolyzed to a greater extent than carbohydrate C. The maltose values are less significant although pointing in the same direction, since other degradation products showing reducing actions may be present but not identified.

TABLE I

COMPOSITIONS OF THE MEDIA USED FOR THE GROWTH OF *B. Coli*

MEDIA	NO. OF DET'NS	pH RANGE	TITRATABLE ACIDITY C.C. 0.1 N NaOH		REDUCTIONS CALC. AS MG. GLUCOSE PER C.C.			
					TOTAL		FERMENTABLE	
			RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
Glucose	14	6.8-7.2	0.2-0.5	0.33	3.96-6.66	5.46	all	all
Maltose	10	6.9-7.3	0.2-0.5	0.28	2.46-5.09	3.86	0.32–0.26	0.06
Lactose	7	6.8-7.3	0.2-0.6	0.29	2.41-4.58	3.19	0.22–0.24	–0.07
A	8	6.8-7.2	0.2-0.6	0.31	1.15-2.48	1.89	0.48- 0.87	0.65
B	11	6.8-7.2	0.2-0.6	0.31	1.66-2.80	2.12	0.19- 0.48	0.35
C	15	6.8-7.2	0.1-0.5	0.31	0.83-1.81	1.38	0.18–0.04	0.06

The intestinal flora of three infants, O, N, and M, were studied. As stated, stool specimens were obtained at two-week intervals for a period of four and one-half months, and *B. coli** strains fished at random and cultured. In the first series, cane sugar was the sole added carbohydrate in the diet, in the second series carbohydrate C was added in place of the cane sugar. In the first series, practically all the strains of *B. coli* were maltose positive. In the second series, for infant M, the dominant strains were maltose negative (twenty-four hours' incubation),† and several of these were cultured. For infants O and N, the dominant strains were maltose positive, but maltose negative strains of these infants, although in the minority, were cultured.

In place of presenting the individual experimental results, the changes due to the growth of the organisms in the different carbohydrate media were calculated and averaged. These average results are given in Table II. The changes due to the growth of the organisms were calculated, not with the average results of the compositions of the media given in Table I, but in each experiment by using the "blank" values (those of the medium without the

*Motile gram-negative bacilli which fermented lactose with formation of acid and gas, methyl red positive and Voges-Proskauer negative. Both coli communis and communior strains were present.

†After forty-eight hours' incubation some fermentation was observed.

addition of organisms) found in that experiment. Fermentable material was absent in all cases after the growth of the organisms so that these results are omitted from Table II.

DISCUSSION

The first fact which appears in these results is the possible variation in any one individual experiment. It is necessary, therefore, to draw conclusions only from a series of experiments and to place limited weight on any one result. Most stress will consequently be placed on the averages.

TABLE II

SOME CHANGES IN MEDIA DUE TO GROWTH OF ORGANISMS

MEDIA	NO. OF DET'NS	DECREASES IN pH		TITRATABLE ACIDITY CHANGES C.C. 0.1 N NaOH		GAS PRODUCED MM.		TOTAL REDUCTIONS. DECREASES CALC. AS MG. GLUCOSE PER C.C.	
		RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
(After feeding cane sugar as additional carbohydrate)									
Glucose	11	2.4-3.2	2.81	0.7-1.0	0.84	2.0-4.1	2.77	1.05-2.22	1.53
Maltose	11	1.8-2.5	2.36	0.1-0.8	0.40	0.7-2.4	1.28	0.23-1.09	0.68
Lactose	5	1.9-2.6	2.36	0.1-0.7	0.42	1.6-2.5	2.28	0.28-0.94	0.53
A	6	1.9-2.5	2.34	0.1-0.4	0.23	1.2-2.3	1.72	0.45-0.82	0.65
B	12	1.5-2.5	1.85	0.0-0.4	0.16	0.5-1.1	0.66	0.27-0.72	0.49
C	11	1.8-2.6	2.20	0.1-0.6	0.33	0.3-1.9	1.21	0.22-0.63	0.40
(After feeding carbohydrate C as additional carbohydrate)									
Glucose	6	2.6-3.2	2.77	0.6-0.9	0.73	2.0-3.2	2.55	1.34-2.56	1.90
Maltose	3	0.1-0.3	0.20	0.0	0.0	0.0	0.0	0.12-0.18	0.15
Lactose	3	2.5-2.8	2.70	0.4-0.6	0.53	1.5-2.1	1.80	0.39-0.91	0.58
A	3	2.1-2.4	2.27	0.2	0.20	1.6-1.9	1.77	0.57-0.77	0.66
B	6	0.5-1.7	1.03	−0.2-0.1	0.02	0.5-1.1	0.82	0.30-0.58	0.40
C	5	−0.3-0.6	0.20	0.0	0.0	0.0-0.3	0.26	0.00-0.20	0.10

The first half of Table II, where the infants had been fed their normal formula with cane sugar as the only added carbohydrate, the pH changes were largest for glucose media, smallest for carbohydrate B media, with the rest in between and much the same. Similar relations were shown for the titrable acidities and gas production. The changes in total reduction were largest for the glucose media, while the rest did not differ much from each other. A comparison of the reduction changes with the compositions of the media given in Table I shows that (a) for the glucose media, about 30 per cent of the glucose was destroyed, the growth of the organisms probably being stopped by the degree of acidity developed; (b) for the maltose media, about 18 per cent of the maltose was destroyed, no glucose being present before or after the culturing; (c) for the lactose media, about the same percentage of the lactose was destroyed but with apparently greater gas production, although the results are not sufficient in number to be entirely convincing; (d) for the carbohydrate A media, all the glucose present originally was destroyed but no other reducing substance attacked; (e) for the carbohydrate B media, all the glucose present originally was destroyed as well as a small amount of other reducing substance, possibly maltose; and (f) for the carbohydrate C media, on the average approximately 30 per cent of the reducing substance, possibly maltose, was destroyed.

The results in the second half of the table where the organisms obtained after C feeding were cultured are average values. As stated before, they refer to maltose negative organisms, the dominant strain of the intestinal flora of one infant and specially selected strains of the intestinal flora of the other two infants. The results for glucose, lactose, and carbohydrate A media were practically the same as in the first half of the table; with carbohydrate B media, there was less breakdown as shown by the pH and reducing action changes, although the gas production was much the same. For maltose and carbohydrate C media there were practically no changes in the tests due to the culture, although the organisms had grown well as shown by the densities. This is in line with what would be expected of maltose negative organisms, while the changes in the carbohydrate B media were evidently due to the glucose present.

To sum up the results presented, so far as the compositions of the carbohydrate preparations used are concerned, C contained no glucose, B contained some, while A contained considerable. In the growing of the cultures in media containing the various carbohydrates, the glucose was used first. If there was no glucose present, maltose (with the maltose positive organisms) and lactose could be utilized to a certain extent. Also, with a small amount of glucose (as in B), after this was used up, maltose was attacked to some extent. The changes in pH, in titrable acidity, in gas production, and in total reductions, in general, ran parallel. The maltose negative strains tested utilized glucose, lactose, and A in the same way as the maltose positive strains, B to a slightly less extent (evidently only the glucose was attacked), while maltose and C (containing no glucose) were not utilized at all.

It is assumed in this discussion that in the breakdown of the starches in forming carbohydrates B and C, maltose and glucose are the only reducing substances formed. It is, of course, possible that other reducing substances, between dextrin and maltose in complexity, are present. The assumption was made for purposes of simplicity in the presentation of the problem and the discussion of the results. The presence of other reducing substances as indicated would change some of the quantitative deductions but not the general relations.

In media with the simple mono- and di-saccharides which were used here the change in pH, the acidity, and the gas formation were as a rule higher than in the mixtures containing carbohydrates A, B or C which are commonly used in infant feeding. An exception to this was carbohydrate A which contains saccharose and more glucose than carbohydrate mixtures B and C, the latter two consisting mainly of maltose and dextrin. Carbohydrate A showed more gas production than maltose. The maltose nonfermenting coli (twenty-four hours' incubation) showed neither acid nor gas production in cultures with carbohydrate C. If the idea presented in the introduction is correct the results suggest the conclusion that carbohydrate mixtures containing no glucose or carbohydrates which readily form glucose might be less apt to cause fermentation in the intestines than those which contain free or readily freed glucose.

Thanks are due Miss Margaret D. Schaffner for carrying out the determinations of the reducing action and acidity.

SUMMARY

The growth of *B. coli* from intestinal flora of infants on media containing different carohydrates was followed by means of changes in pH, total acidity, and total and fermentable reducing actions, and the gas evolved. The carbohydrates used included glucose, maltose, lactose, and three commercial preparations, i.e., a syrup, and two mixtures obtained by the partial hydrolysis of starch.

B. coli isolated from infant stools produces somewhat more gas from glucose than from lactose, considerably more gas from these two carbohydrates than from maltose. Of three commercial carbohydrate mixtures the one containing glucose and saccharose showed more gas production than those consisting simply of maltose and dextrin. Acid production showed differences of a similar order but to a very much less extent. Some of the *B. coli* strains did not attack maltose in twenty-four hours and thus showed still greater differences of the same nature.

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A FURTHER CONTRIBUTION TO THE LIVER-KIDNEY SYNDROME*

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IN PREVIOUS publications,[1] we have called attention to an apparently characteristic train of clinical symptoms and pathologic changes which began with hepatic damage and were followed by serious parenchymatous kidney degeneration. We have called this clinical and pathologic relationship between the liver and the kidney a "liver-kidney syndrome." Clinical and pathologic experimental study of this syndrome seemed to justify the assumption that its pathogenesis consisted essentially of a primary parenchymatous hepatic damage. This damage appears to be independent of both infection and ordinary cellular autolysis, and is followed by an elaboration of certain undetermined hepatogenic toxins or metabolites which act specifically upon the kidney tubules and by that action produce a severe and often fatal clinical picture terminating in many instances in a condition resembling uremia.

It has been suggested by Bartlett[2] in reviewing our cases that the kidney findings are those of acute nephritis due to infection in the liver. The question as to whether bacterial toxins or metabolites toxic to kidney parenchyma may be the important factors producing the kidney damage in the liver-kidney syndrome is, of course, highly speculative. In all of our cases, particularly those associated with gallbladder infection, we have attempted to eliminate infection as a factor by pre- and postmortem bacteriologic studies. We have observed the syndrome not only in cases of uncomplicated hepatic trauma, but also in metastatic carcinoma of the liver. In neither of these could cellular or cultural evidence of infection be demonstrated.

It has also been suggested that the etiologic factor in the production of the kidney changes in our cases has been a specific toxin produced by the liberation of autolytic products of aspetic tissue necrosis similar to that seen in extensive burns and in pancreatitis with fat necrosis. In answer to this, it should be mentioned that aside from the hemorrhagic diathesis, so frequently an important clinical feature in the syndrome, no other parenchymatous organs show the changes at necropsy suggestive of such a process. In the toxicities of autolytic tissue necrosis, submucous hemorrhages are extremely rare. Moreover, the characteristic chain of clinical manifestations with the gradually increasing oliguria, low grade edema, uremia and the progressive increase in blood nitrogen are certainly not usual findings in this type of toxicity.

Classification of the Renal Lesion.—Although there is considerable difference of opinion regarding the classification of the nonsuppurative types of

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Received for publication, May 14, 1935.

nephropathies, we have considered the kidney lesion in our liver-kidney syndrome to be essentially a nephrosis. We base our classification upon the fact that the anatomic changes are chiefly degenerative rather than inflammatory. Whatever the cause may be, the result is a degenerative and not an inflammatory lesion of the kidney. There is, in fact, evidence other than our own that such kidney changes may occur with or without the presence of bacteria. Thus, hepatonephrosis is almost constantly present in yellow fever where, not infrequently, the renal changes are in many ways similar to those found in our cases. On the other hand, an almost identical kidney tubular lesion is found in certain cases of chemical poisoning. In both instances, the assumption seems justified that the degenerative kidney change is not a result of direct infection but rather that of a powerful toxin. Certainly in the renal lesions of the cases we have studied, there is no evidence of glomerular nephritis; the lesions are almost wholly confined to the tubular epithelium and the interstitial tissues are apparently undergoing degenerative changes.

We have attempted to demonstrate glomerular alterations by Mallory's analine blue and Masson's polychrome stains, which stains failed to reveal any of the characteristic anatomic alterations in the glomeruli characteristic of any stage of glomerular nephritis. Although some evidence of endothelial swelling was seen at times and in some specimens prominence of the epithelium was apparent, control specimens taken from the kidney of a perfectly normal young woman, who died ten minutes after being struck in the jugular vein by a jagged piece of automobile windshield glass, showed the same degree of these glomerular changes. Moreover, these stains did not show any of the characteristic hyaline bundles of glomerular nephritis in the glomeruli of any of our cases, and pus cells were never numerous while red blood cells were always present, at times abundantly so.

Although focal interstitial infiltrates consisting almost exclusively of mononuclear cells were often present, it is our opinion that these represented a reaction to degeneration. The supposition that the presence of red blood cells in the urine indicated an acute glomerular nephritis can be controverted by the fact that in all instances in which red blood cells were found in the urine, we were able to demonstrate submucous petechiae in the kidney pelves and bladder; and we also always found microscopic focal hemorrhages in the medullary portion of the kidneys.

The pathologic picture of the kidneys in our cases bore no similarity to that of so-called lipoid nephrosis. We were able to reach this conclusion, since in no instance did either differential fat stains or polarized light reveal evidence of fatty degeneration or infiltration.

The changes found in the kidneys of the cases under discussion were in many important details very similar to the nephrosis found in certain cases of chemical poisoning, in pregnancy toxemias, and in other true advanced toxic nephroses.

In summarizing the histologic picture of the changes characteristic of the nephrosis in the liver-kidney syndrome, it is to be noted that the principal

finding is degeneration of the tubular epithelium in the more highly differentiated tubules, the degeneration ranging from a minor grade of cloudy swelling through vacuolar degeneration to actual epithelial necrosis. Although, as mentioned above, minor evidence of glomerular capillary and epithelial irritation was not infrequently seen, this was always of minor importance.

Another striking feature was the focal medullary cellular infiltration. Interstitial hemorrhages were very frequent and in many instances pigment-laden phagocytes filled with hemosiderin were found in these focal areas. Moreover, the cellular components of these infiltrates were almost exclusively round cells, eosinophiles and plasma cells, and every feature of the reaction suggested a reaction to degeneration.

CASE REPORTS

As a further contribution to the liver-kidney syndrome which we have described, we wish to present two cases, one of pure trauma where sections were taken from the liver in areas both adjacent to and within the pulpified region, and also throughout various parts of the nontraumatized liver. In none of these was there the least evidence of any cellular reaction which could be interpreted as the result of bacterial invasion. Our other case is one of pure liver infection in which multiple suppurating thrombi were found in the portal venous branches. In this case the clinical picture was that of progressive anuria and uremia with extensive blood nitrogen retention and almost exsanguinating mucous surface bleeding. At necropsy, the kidney showed the anatomic findings identical with those seen in the traumatic case where no evidence of any infection could be demonstrated.

CASE 1.—*History:* Shortly before admission to the hospital, the patient, a well-developed man of sixty-one years of age, was in an automobile accident. He walked into the hospital complaining of pain in his right chest and his right knee. Physical examination demonstrated a fracture of the right patella and the first to tenth ribs of the right anterior thorax and second and third ribs of the left thorax. His temperature was 99° F., his pulse 90. He had, previous to this accident, enjoyed excellent health all of his life.

Approximately twelve hours after his admission to the hospital, his temperature rose to 100° F., his pulse to 160, while his respiration remained normal. Complete laboratory examinations including urine, blood count, and blood chemistry were normal. His general condition remained about the same except for a moderate abdominal distention until the next day (the second day of his illness) when his abdominal distention increased, his temperature rose to 101° F., and he began to vomit small quantities of blood and pass large quantities of blood by rectum. On physical examination, tenderness was present over the entire right upper quadrant of the abdomen. He rapidly became toxic with occasional periods of muttering delirium. Despite the intravenous and subcutaneous injection of considerable quantities of glucose and saline, his urinary output rapidly decreased. Slight edema of the ankles and back appeared. Examination of the urine revealed the presence of a faint trace of albumin, hyaline casts and microscopic blood.

The next day (the third day of his illness) he became delirious, abdominal distention increased, and he vomited considerable quantities of blood. His bowels were incontinent and consisted almost entirely of blood. His temperature rose to 102° F., and his pulse remained at 160. His urinary output decreased to almost complete anuria but examination of small quantities revealed a heavy trace of albumin, 30 to 40 pus cells per high power

field of centrifuged urine, hyaline and granular casts, and microscopic blood. His blood count was normal, but his blood chemistry showed a nonprotein nitrogen of 75 mg. and a creatinine of 3 mg. per 100 c.c. His blood sugar fell to 58.8 mg. per 100 c.c.

From muttering delirium he passed into a deep coma and died in a state clinically resembling uremia.

Necropsy: Surface inspection of the body revealed numerous scratches and bruises and there was a faint lemon tint to the skin. In the peritoneal cavity, some clear brownish fluid was present. Over the dome of the liver, a large mass of clotted blood was found and three cracks were present in the capsule. The deepest rent was found immediately to the right of the falciform ligament and this extended almost entirely through the liver. Lateral to this fracture, the hepatic parenchyma was pulpified and of a deep brick red color. This zone of pulpification extended from 2 to 4 cm. into the liver substance all along the line of fracture. There was likewise considerable extension of the hemorrhage into the perihepatic

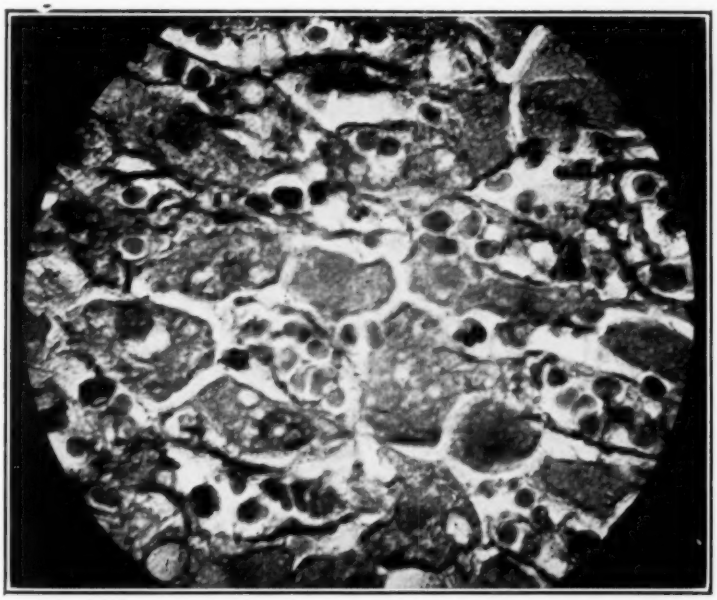

Fig. 1.—Photomicrograph of the liver in Case 1 taken through border of necrotic zone; observe anuclear liver cells and lack of inflammatory reaction.

retroperitoneal tissue. The remaining liver parenchyma was not involved in the hemorrhage and showed a pale, swollen, somewhat mottled surface. The liver weighed 1,400 gm.

The kidneys were somewhat swollen. The capsules stripped with ease and small pinpoint hemorrhages were seen in the medullary zones of both kidneys on cross-section. The markings were very indistinct and the cut surfaces were pale and somewhat bulging. The ureters were negative. The bladder was contracted, and there were about 5 c.c. of urine present which contained albumin, white cells, some granular casts, and microscopic blood.

In the chest cavity, nonperforating fractures of the first ten ribs of the anterior right thorax were found, and the second and third ribs on the left side were cracked. Some hemorrhages were found under the epicardium of the left auricle and interstitial hemorrhages were present in both lungs.

There was considerable interstitial hemorrhage into the capsule of the right adrenal.

Histologic Pathology: Sections through the liver adjacent to the area of fracture and pulpification showed a zone of extensive hemorrhage in which masses of fibrin were buried. Here and there a few scattered necrobiotic leucocytes were found. The adjacent liver tissue showed varying stages of necrosis, ranging from complete epithelial disintegration through

hyaline anuclear necrosis (Fig. 1) to varying degrees of parenchymatous degeneration. At the outer border of the zone of trauma and extending a considerable distance into the grossly unaltered hepatic parenchyma, focal interstitial hemorrhages were present, and the liver cells were quite intact except in these small focal areas. In the zone of pulpification, wide expanses of liver tissue were observed where the hepatic cells possessed no nuclei but still retained their outlines. There the sinusoids were intact and in most instances plugged with masses of red blood cells. There was no evidence of any inflammatory reaction, such as would be produced by an infection, in any of the sections viewed.

The kidneys showed different stages of cloudy swelling, vacuolar degeneration and in the more highly differentiated tubules there was even loss of nuclei in many cells. Many of the tubules contained masses of stringy albuminous material or casts. The glomeruli were perhaps slightly more cellular than normal, and between the tuft and the glomerular capsule occasionally thin stringy strands of albuminous precipitate were seen, but for the most part the glomeruli were relatively normal in appearance (Fig. 2). In the medullary portion of the kidney, small focal areas of hemorrhage were encountered as well as areas wherein large

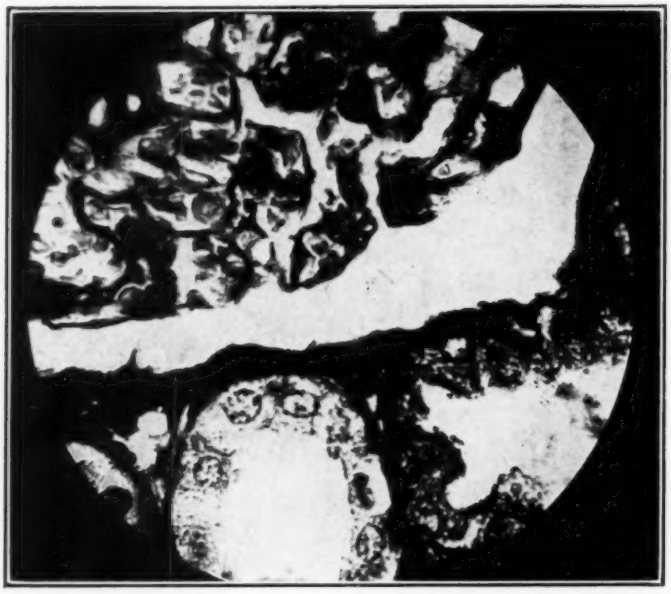

Fig. 2.—Photomicrograph of the kidney in Case 1; note very minor degree of glomerular change (Mallory's analine blue stain).

numbers of phagocytes heavy laden with pigment were seen. These large pigment cells were bulging with deep brown hemosiderin granules. Occasional scattered round cells were likewise seen in these foci. Many of the tubules in the medullary portion likewise contained albumin or casts. Special stains for the glomerulus showed only minimal changes.

The mucosa and submucosa of the stomach showed extensive hemorrhage and some edema. Scattered polymorphonuclear leucocytes and round cells were present in the stroma.

The other organs revealed nothing particularly noteworthy histologically.

CASE 2.—*History:* The patient was a well-developed farmer forty-two years of age. He had been perfectly well until four days before his admission to the hospital. Although he had not seen a doctor until just before his admission, he gave a history of symptoms typical of appendicitis. His physical findings were those of a perforated appendix with local peritonitis. His temperature was 100° F., his pulse 100, and his respiration 30. His blood picture was normal except for an increase in leucocytes to 11,000. His urine was highly concentrated and contained a trace of albumin; otherwise, it showed no abnormalities. The

following day, the fifth day of his illness, he became lightly jaundiced and passed a considerable amount of dark blood by rectum. The next morning his jaundice had deepened and he began to bleed from his mouth and lips. He also coughed up blood-stained sputum and he continued to pass blood by rectum. At this time his urinary output decreased rapidly to almost complete anuria. The urine contained albumin 1-plus, 20 to 30 red blood cells to the high power field, and an occasional pus cell. His blood count showed 4,320,000 red cells, 8,250 leucocytes with 80 per cent polymorphonuclears and 20 per cent small lymphocytes. Blood chemistry determinations revealed a nonprotein nitrogen of 150 mg., creatinine 3.7 mg., chlorides 330 mg., and blood sugar 118 mg. per 100 c.c. of blood. His temperature rose to 101° F., pulse to 110, and respiration to 35. During the next twenty-four hours, the seventh day of his illness, the bleeding continued from his mouth, lips, lungs, and intestinal tract. The jaundice remained practically unchanged. The urinary output further decreased until it was difficult to get a sufficient sample for examination. The urine contained a trace of albumin, 20 to 30 red cells to the high power field, granular casts, a small amount of bile, and an occasional pus cell in the uncentrifuged specimen. The blood nonprotein nitrogen rose to 285 mg. and the creatinine to 4.5 mg., the blood sugar fell to 51.2 mg. and the blood chloride increased to 430 mg. per 100 c.c. of blood. The blood count at this time showed 2,150,000 red cells and 11,800 leucocytes with 72 per cent polymorphonuclears and 28 per cent lymphocytes.

Toward the latter part of the seventh day of his illness, the patient died with symptoms suggesting uremia.

Necropsy: Both clotted and unclotted blood were found in the mouth and nose. The skin was jaundiced. The abdomen was distended. When the abdominal cavity was opened, the gutter along the cecum and ascending colon was filled with flocculent, semipurulent, bloody exudate and the cecum and ascending colon were distended, their walls greatly thickened and indurated and of a deep bluish black color. The mucosa was extensively ulcerated and a gangrenous appendix was found lying in a walled-off pool of foul-smelling exudate.

The liver was large and hard and on section pale and shining. When pressure was applied to it, focal pinpoint to pinhead size dots of milky pus appeared in clusters on the cut surface. The superior mesenteric and portal veins were filled with semiautolytic suppurating thrombi. The stomach and entire bowel contained a large amount of bloody feces. The kidneys were greatly enlarged, their capsules tense, and on section the renal parenchyma bulged out under the incised capsule. The markings between the cortex and medulla were very indistinct. Both kidneys were definitely jaundiced. Numerous petechial hemorrhages were visible in the pelves. Both lungs showed subpleural and interstitial hemorrhages, and edema and terminal bronchopneumonia was present in each lung.

Histologic Pathology: The liver showed a rather diffuse, low grade, parenchymatous degeneration. There was considerable swelling and even desquamation of many of the Kupffer cells and scattered here and there throughout the sinusoids leucocytes were found. In the portal areas, a diffuse infiltration with round cells and scattered polymorphonuclear leucocytes was seen. In some of the very smallest portal venous channels, masses of suppurative thrombi were seen (Fig. 3). Moreover, in the larger portal ramifications, purulent necrotic exudate was found and in some areas there was considerable fragmentation of the vein walls. However, very little extension of the exudate had taken place into the liver proper. Under the higher magnification, there was no evidence of obstructive jaundice except occasionally near the center of the lobule where the parenchymatous cells contained scattered deposits of brownish pigment and in some of the small bile canaliculi minute bile thrombi were present. The liver cells in many instances had quite indistinct outlines and showed varying degrees of nuclear lysis. Many of the Kupffer cells, in addition to being tremendously swollen and desquamating, contained some pink semigranular material of undetermined character. There were definite hemorrhages in the gallbladder mucosa.

The kidneys showed striking tubular changes, the most interesting of which was an extensive degeneration. This change varied from low grade cloudy swelling to frank epithelial necrosis. In some sections, focal leucocytic infiltrations, in which either round cells or plasma

cells predominated, were scattered in the medulla and cortex. In addition to these cells, pigment containing phagocytes and other evidences of previous interstitial hemorrhage were apparent. Fresh interstitial hemorrhages were also noted in the medullary interstitial tissue.

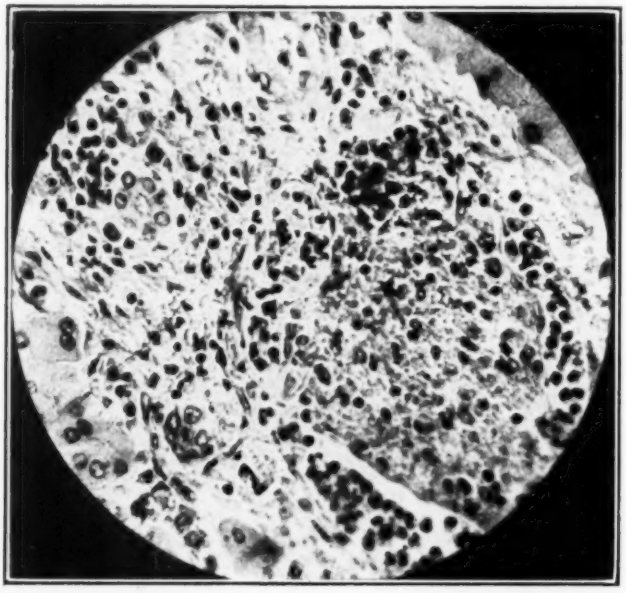

Fig. 3. Photomicrograph of the liver in Case 2, showing a thrombus in a small portal radicle and interstitial pus cell infiltration.

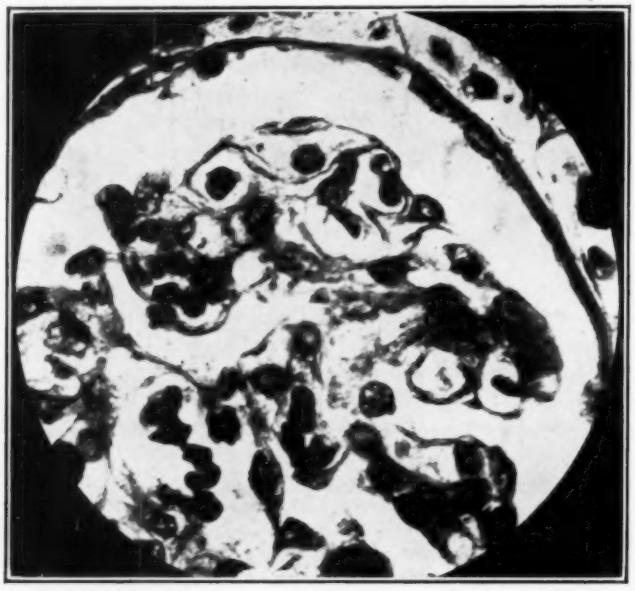

Fig. 4.—Photomicrograph of kidney glomerulus in Case 2; glomerular changes are negligible (Mallory's analine blue stain).

Many of the tubules where the necrosis was not so marked showed a generous accumulation of albuminous precipitate in their lumina. In other areas the outlines of the tubular epithelium were so irregular and indefinite that one gained the impression that most of the

cells were beginning to slough away. In many tubules, hyaline droplets were present in enormous numbers. Special stains were employed for glomerular epithelium and basement membrane, however. With these, the glomeruli showed but minor changes consisting of a low grade swelling of the capsular epithelium and some proliferation of the endothelium (Fig. 4). Occasionally some of the tubular cells had a faint brownish tinge suggesting imbibition of bile, but this was by no means striking.

The lungs showed edema, early bronchopneumonia, and in the edematous fluid large numbers of red cells in varying stages of hemolysis could be seen.

The appendix showed the typical picture of acute suppurative appendicitis with suppurative thrombi in the venous channels and beginning gangrene of the cecal wall.

Discussion of Cases.—We consider the following points in Case 1 important in the proper evaluation of the syndrome. First, there was no evidence of infection. Second, although the patient was faintly jaundiced toward the end of his illness, there was no microscopic evidence of bile pigment in the renal epithelium. It is also interesting that the area of fracture and hepatic pulpification was in the middle of the liver between the right and left lobes. This location of the pulpification was similar to that observed and previously reported by us in the liver of a patient who presented this same syndrome. It should be emphasized that Case 1, prior to the accident, had been a well and apparently normal individual and that the first clinical manifestation of renal impairment appeared on the third day after the injury. The kidney lesion was predominantly tubular in character, the changes being those of varying degrees of parenchymatous degeneration with but few minor evidences of glomerular damage. Although some old foci of glomerular fibrosis were present, these changes were not inconsistent with the common findings in the kidneys of persons of this same age dying from some totally unrelated disease. This latter finding, therefore, was considered unimportant. Clinically, the patient vomited blood and passed blood per rectum, and at necropsy submucous hemorrhages were present in the gastrointestinal tract. This gastrointestinal bleeding is very frequent in these cases and, when present, is one of the outstanding features of the syndrome. Such bleeding is prominent not only in the traumatic cases, but has also been observed in the great majority of cases in which the fatal syndrome has followed other forms of liver damage.

In sharp contrast to Case 1, two factors were present in Case 2 which might be considered as of possible influence in the production of the marked renal lesion found at necropsy; i.e., infection and jaundice. We have included Case 2 because we wish to show that the renal picture produced by pure hepatic trauma, unassisted by either jaundice or infection, may produce the same clinical and pathologic picture as that observed in cases presenting the liver-kidney syndrome which are not associated with infection.

In Case 2, the liver abscesses were, for the most part, of microscopic dimensions; in only a few areas could they be recognized with the naked eye. When pressure was applied to the liver, minute droplets of pus oozed out on the cut surface. Histologically, these were found to be small portal vein thrombi surrounded by areas of advanced parenchymatous degeneration proceeding in many regions nearly to necrosis of the hepatic cells. The patient in Case 2 was considerably jaundiced, and grossly and microscopically the

kidneys showed some staining with bile. With the exception of this addition to the gross and microscopic picture of the kidney, the renal changes were in all respects comparable to those found in the nonjaundiced, noninfectious case of traumatic liver pulpification. Moreover, clinically, aside from the symptoms of acute appendicitis and ascending pylephlebitis with jaundice, the sequence of clinical manifestations such as gradually increasing oliguria proceeding to anuria, rapidly mounting blood nitrogen and extensive bleeding from the mouth, stomach and bowel, duplicated in all respects those findings observed in the traumatic case.

The renal lesion found in these cases was very interesting. The glomeruli showed but minor alterations; in some instances slight accumulations of albumin had occurred in the capsular space, while in others there was a slight epithelial proliferation and endothelial swelling as well as a low grade thickening of the glomerular membrane. These changes, however, were minimal and negligible when compared to the remarkable degenerative changes found in the tubules.

The classical picture of bile nephrosis so frequently encountered in obstructive jaundice was duplicated in the renal picture in certain phases only. Fahr[3] mentions the presence of fatty changes in the renal epithelium in addition to the universal or streaked distribution of bile pigment in the tubules, Bowman's capsule, and intratubular albumin. In only one case in his experience was there evidence of localized inflammatory lesions. Our Case 2 showed bile only in minor quantities and the impression was gained from careful microscopic study that something aside from the infiltration of bile was responsible for the overwhelming tubular damage.

The presence of occasional small foci of round cells and plasma cells in the medullary portion of the kidney was considered by us to be evidence of a reaction to a degeneration because granular débris was present in these foci although no pus cells were found. The presence of large numbers of cells bulging with hemosiderin pigment indicated that these cellular elements represented a reaction to a degenerative process which had doubtless been accompanied by some degree of hemorrhage. This latter hypothesis was substantiated by the finding of other fresher interstitial hemorrhages into which no leucocytes had as yet wandered.

Literature.—In a previous report, we recorded several cases, similar to those presented here, which had already been reported in the literature but in which no attempt had been made to explain the apparent relationship between the liver and the kidney.

McKnight[4] reported the case of a healthy boy nineteen years old who suffered a rupture of the superior surface of the right lobe of the liver as a result of an automobile accident, and at operation the rent in the liver was packed in the usual manner. Postoperative convalescence was uneventful until the sixth day when the patient became nauseated, jaundiced, and developed generalized abdominal distention. Laboratory findings at this time demonstrated a blood urea of between 240 and 270 mg. per 100 c.c. of blood. Albumin and granular and hyaline casts appeared in the urine. The leucocyte

count during this time ranged as high as 72,000. After three weeks of symptoms highly suggestive of uremia, the patient recovered but on dismissal still showed a trace of albumin and a rare cast in the urine. This history suggests a parallel to the histories in our syndrome cases.

Also, in 1931 Wulsten[5] observed an eleven-year-old schoolgirl who suffered a trauma to the liver while riding on a sled. Some time following the injury she bled from the nose and vomited blood. She became progressively anemic and red cells appeared in the urine. The red cell count and hemoglobin continued to decline until the hemoglobin was 19 per cent and the red count 1,500,000. After a rather long illness, the patient died. At necropsy, although the capsule of the liver was intact, a large cavity was present which involved almost the entire right hepatic lobe and which was filled with dirty brown fluid. Multiple subpleural and subepicardial ecchymoses were present and many punctate hemorrhages were seen in the mucosa of the bowel and in the kidney pelves. No histologic record of the kidney was given. In the liver, there was no microscopic evidence of infection but necrotic foci were found in the liver tissue surrounding the cavity. It was Wulsten's opinion that the mucous surface bleeding may have been due to absorption of toxic products from the necrotic liver tissue. Unfortunately no blood chemical studies were mentioned.

These two cases are presented only as additional circumstantial evidence. Further research of the literature of the liver and kidney shows that, although cases of this general character have not frequently been recorded, they may not be rare. This is brought out in a report by Stanton[6] who analyzed the cause of 500 deaths following gallbladder operations. In this series, he noted that twenty-four died of renal failure, chiefly anuria.

In 1927, Wilensky and Colp[7] published their observations regarding the retention of nitrogen bodies in cases of biliary tract disease. They noted that in ordinarily mild or moderately severe cases, disturbances in nitrogen metabolism were not observed prior to operation. They also mentioned that such patients were frequently not operated upon until the essential lesion was relatively far advanced, so that nephritis (renal epithelium degeneration) was frequently present at the time of observation.

Since we called attention to this syndrome as a definite clinical and pathologic entity, other observers have reported cases of similar character. Thus, Cole[8] of Washington University, in a recent discussion of hepatic insufficiency, cites the case of a woman forty-eight years old who, from a clinical standpoint, appeared to be a good operative risk. Cholecystogastrostomy was performed for common duct obstruction. A few days after the operation she developed edema of the ankles, and albumin and large numbers of granular casts appeared in the urine. There was a progressive oliguria which finally terminated in total anuria. The nonprotein nitrogen rose to 60 mg. per 100 c.c. of blood, she was drowsy and irrational, and died on the ninth postoperative day. At necropsy, the liver showed extensive cellular necrosis and atrophy while the kidneys presented only moderate parenchymatous changes.

Hemorrhages were present in the walls of the renal pelves, and some parenchymatous hemorrhages were likewise seen.

It is Cole's opinion that infection played little part in the hepatic insufficiency and he likewise stated that he considered that an associated kidney damage in cases of severe hepatic disease to be extremely common and important.

Even more recently Eiss,[9] in a discussion of conservation of hepatic function in gallbladder operations, states that in his experience in biliary tract surgery he has observed "several cases of postoperative death without infection or hemorrhage with rapidly rising fever, rapid pulse and the terminal uremic syndrome." He is convinced that these cases are much more frequent than the number of reported cases would indicate and "that the tragedy of these deaths is that they occur in patients who appear clinically to be good operative risks."

Similarly, Heyd[10] in an editorial in *Surgery, Gynecology and Obstetrics* describes a type of "liver-death" as follows:

> Previous to operation these patients have had what was considered normal kidney function and no question was in the mind of the surgeon as to the competence of the kidney to carry on its function in the presence of an operative intervention. Forty-eight hours after an operation on the gallbladder or common duct, the patient quite rapidly presents the picture not dissimilar to shock, with cold, clammy skin, gradual failure in water elimination and a rise in urea nitrogen. The urinary output becomes less and less, and a mild delirium develops with increased frequency of pulse and temperature, and finally coma and death. These patients are not jaundiced either before or after operation, and there is a distinct interval of apparently normal postoperative conduct of from twenty-one to thirty-six hours between the operation and the onset of the terminal clinical picture.

Previous to the publication of our first article,[1] Heyd[11] had considered this type of "liver-death" as a condition of hepatic exhaustion related in some way to the pancreas.

Theories of Toxin Pathogenesis.—Our cases in which the liver-kidney syndrome appeared following traumatic fracture of the liver now are two in number; one was reported in an earlier paper. In both instances, as mentioned above, the traumatic lesion was situated in the right lobe near the midline and was so placed as most easily to cause trauma of the main intrahepatic branches of the hepatic artery.

That extensive hepatic necrosis, even at the site we have discussed, may likewise fail to cause fatal kidney changes can be illustrated by the case reported by McKnight.[4] There seems little doubt that his patient recovered from a definite liver-kidney syndrome, but it is significant that three weeks later the patient continued to show albumin and casts in his urine, showing that very definite kidney damage had been effected.

Recently we have been fortunate in that Dr. T. G. Orr has permitted us to observe two cases of traumatic liver necrosis which were subjected to laparotomy and in which the clinical and blood chemical findings showed all of the features of the liver-kidney syndrome except the final anuria. These two patients recovered and now have normal kidney functions; these cases will be reported by Dr. Orr.

We have also seen several cases in which, during an acute exacerbation of chronic cholecystitis, albumin, red cells and casts appeared in the urine and a rise occurred in the nonprotein blood nitrogen, in one instance to 65 mg. per 100 c.c. of blood. These patients have recovered with apparently normal kidney function. Incidentally, we have likewise observed cases which, following cholecystectomy, showed minor evidences of kidney damage and have subsequently recovered.

In this syndrome many puzzling and apparently contradictory points are brought to mind. From our clinical and experimental observations, it does not appear possible that the toxin is the product of pure hepatic necrosis since, in almost all of the cases in which the liver-kidney syndrome appeared, hepatic necrosis except in the traumatic cases was not a striking finding. Moreover, in experimental ligation of the hepatic artery as well as in experimental hepatic trauma with packing of the central portion of the liver, we have found that the most extreme degrees of hepatic necrosis were, in many instances, not followed by nitrogen retention or kidney damage, while in occasional instances definite tubular degeneration and nitrogen retention were observed although liver necrosis of a very much less degree had occurred.

In the human being, ligation of the hepatic artery has been performed in a number of instances. Recently, Shann and Fradkin[12] recorded such a case in which massive infarction took place in the right lobe. This infarct, measuring 10 by 3 by 5 cm., was removed at a later operation. No mention of any renal complication was recorded. Several cases of traumatic liver necrosis with massive sequestration are mentioned in the literature. For example, Biernath and Fertig[13] record a case in which the entire left lobe of the liver became necrotic and was surgically removed. In this case, likewise, no kidney complication was mentioned.

Many important things are known regarding the rôle of the liver in protein metabolism, and the remarkable detoxifying powers of the liver against both endogenous and exogenous poisons have been demonstrated many times. Thus, Rolleston and McNee[14] mention the frequent association of a severe renal insufficiency with retention of nitrogen bodies in the blood in cases of severe hepatic disease.

It is interesting in this regard that in certain cases of Eck fistula, when part of the portal circulation had been shunted into the general circulation and high protein diets were instituted, severe and even fatal toxemias have occurred. Moreover, it has also been demonstrated that a high protein diet has resulted in severe or even fatal toxemia in dogs that have had large portions of their portal blood shunted back into the general circulation as the result of the liver cirrhosis produced by carbon tetrachloride.

These observations seem to suggest the idea that the pathogenesis of the liver-kidney syndrome depends primarily more upon the development of some specific type of intracellular hepatic damage than upon the degree of actual morphologic cellular damage to which ordinary cell injury may extend.

When we consider the wide variety of liver lesions and the more constant sequence of clinical events in which the renal picture assumes the most promi-

nence as the syndrome progresses, it appears more and more convincing that some toxin is the causative factor. This toxin may have been produced as the result of a perversion of function of damaged liver cells or by a lack of some physiologic detoxifying function of the liver parenchyma which permitted the production or the accumulation of a substance highly toxic to renal function.

The occurrence of hemorrhages in severe liver disease has been a frequent observation for a half century, but in most instances it has been attributed to a delayed coagulation time resulting from icterus. As far back as 1880, Schiff[15] noted that there occurred changes in the coagulation time which he felt were etiologic factors in the "hemorrhagic diathesis" associated with hepatic disease with and without icterus. The clotting time was found by Mann and Bollman[16] to be first decreased and then increased in total hepatectomy in dogs, and he observed marked oozing from all the abraded surfaces in those animals which had been kept alive for some hours. The clotting time may remain normal even though fibrinogen may be decreased although, as Whipple and Foster[17] have shown, the clots may be so delicate that they will not stop hemorrhage. Mucous surface hemorrhage has been one of the particularly puzzling complications of the liver-kidney syndrome and in many instances this has been one of the most striking features in the clinical picture. Whether this is due to hepatic damage or renal insufficiency resulting from the action of some toxin which directly affects the capillary endothelium, is a matter of conjecture. Rolleston and McNee[18] have observed general hemorrhages in the late stages of hepatic cirrhosis, and they believe that "when the liver has become incapable of stopping poisons absorbed from the alimentary canal," a state of general hepatic toxemia results with a low blood fibrinogen content with subsequent damage to the blood vessels. They have seen petechiae of the skin and bleeding from the mucous membranes of the mouth and gums. In one case recorded by Webber,[19] hemorrhage took place even in the external ears, in addition to hemoptysis and bleeding from the gums. Rolleston and McNee[20] likewise cite a case recorded by Leudet where hemorrhage took place from the external ear in cirrhosis. A hemorrhagic diathesis is also not a rare complication in certain cases of renal disease where no accompanying liver lesion is present. At present, however, the true etiology of such bleeding is not completely understood.

CONCLUSIONS

1. A brief description of the liver-kidney syndrome is outlined and questions of etiology are discussed.

2. An attempt is made to classify the renal lesion in this syndrome as a toxic nephrosis.

3. Two illustrative cases of the liver-kidney syndrome with complete necropsy studies are recorded and special microchemical studies of the kidneys are described. These two cases are recorded in an attempt to illustrate certain theories regarding the possible pathogenesis of the liver-kidney syndrome.

4. Additional cases are recorded from the literature which may be examples of the liver-kidney syndrome.

5. A theory is advanced to account for the elaboration of an hypothetical toxin believed to be responsible for the renal damage in the liver-kidney syndrome, and mention is made of experiments in which uniform results could not be obtained when an attempt was made to reproduce the liver-kidney syndrome in animals.

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THE INTRANASAL APPLICATION OF INSULIN*

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SINCE the discovery of insulin investigators have attempted to introduce it into the body by various methods. Woodyatt[1] in 1922 said "experiments were conducted with oral, rectal, vaginal, intranasal, intravenous, and subcutaneous administrations. Inunctions were also tried. Many variations were attempted in connection with each. Positive effects were obtained with subcutaneous and intravenous injections, very weak, doubtful or frankly negative results with the others." Up to the present time intravenous and subcutaneous administrations have been employed to the exclusion of all other methods.

Telfer[2] in 1923 reported that insulin could be introduced into the blood stream by means of inunction. Harrison[3] in 1926 repeated this work and found that the inunction of insulin was useless even in very large doses. Peskind, Rogoff and Stewart[4] in 1924 found that insulin when injected per rectum into rabbits was absorbed and produced lowering of the blood sugar; in dogs, negative results were obtained. Heubner, de Jongh and Laquer[5] in 1924 described the lowering of blood sugar in diabetic patients by inhalation of insulin. Fisher[6] in 1924 found some absorption of insulin by the intestine, vagina, and scrotal sac. Gänsslen[7] in 1925 described lowering of the blood sugar in diabetic patients by inhalation of an insulin spray. Miller[8] in 1926 reported that insulin given in absolute alcohol or 95 per cent alcohol solution, in keratinized capsules, lowered the blood sugar level of diabetic patients. Stephan[9] in 1929 described lowering of blood sugar following the administration of insulin by mouth. This work was not confirmed by Wahneau and Bertram[10] or by Bertram, Horwitz and Wahneau.[11] Bollman and Mann,[12] working with intestinal catheters and with ileac loops, found that large amounts of insulin might be instilled into the duodenum, jejunum, or ileum without any appreciable effect on the blood sugar of normal dogs. Similar results were found with administration into the ileac loop.

A summary of these results bears out the initial statement of Woodyatt that with methods other than subcutaneous or intravenous injection "very weak, doubtful or frankly negative results" have been obtained.

Recently we have repeated some of these experiments with variations, and, in the course of our work, studied the problem of intranasal absorption. We have obtained undoubted evidence of the activity of insulin, either when sprayed or when instilled into the nostrils in normal rabbits, normal dogs and in diabetic patients, under certain conditions.

Preliminary experiments indicated that the instillation or insufflation of insulin in the nose produced either frankly negative or doubtful results. We studied next various solutions which might possibly increase absorption

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Received for publication, May 23, 1935.

TABLE I

DATE	ANIMAL	TIME	BLOOD SUGAR MG.
3/15/35	Rabbit No. 1	8:50 A.M.	247.0
		8:55 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		10:28 A.M.	177.0
		11:47 A.M.	149.0
3/15/35	Rabbit No. 2	9:05 A.M.	105.5
		9:08 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		10:40 A.M.	62.5
		12:40 P.M.	55.2
3/20/35	Dog No. 1	9:50 A.M.	96.0
		9:55 A.M.	0.4 c.c. insulin-ethylene glycol mixture (200 units) intranasally
		10:50 A.M.	58.8
		11:45 A.M.	58.3
		12:45 P.M.	44.2

The effect of this solution when applied intranasally in diabetic patients is shown in the following:

DATE	PATIENT	TIME	BLOOD SUGAR MG.
3/18/35	No. 1	7:30 A.M.	150
		8:15 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		9:15 A.M.	149
		10:15 A.M.	122
		11:15 A.M.	83
		12:15 P.M.	110
3/19/35	No. 2	7:30 A.M.	135
		8:15 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		9:15 A.M.	87
		10:15 A.M.	94
		10:15 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		11:15 A.M.	69
		11:15 A.M.	Slight reaction
		11:30 A.M.	More marked reaction
		11:40 A.M.	Orange juice
		12:15 P.M.	115
3/21/35	No. 3	8:00 A.M.	225
		8:50 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally (spray)
		9:50 A.M.	197
		10:50 A.M.	172
		11:50 A.M.	148
		12:00 NOON	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally (spray)
		1:00 P.M.	137
		2:00 P.M.	116
3/21/35	No. 4	8:00 A.M.	256
		8:50 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally (spray)
		9:50 A.M.	154
		10:50 A.M.	125
		11:50 A.M.	115
3/29/35	No. 5	8:30 A.M.	188
		9:30 A.M.	180
		9:40 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		10:30 A.M.	144
		11:30 A.M.	142

TABLE I—CONT'D.

DATE	PATIENT	TIME	BLOOD SUGAR MG.
3/30/35	No. 6	12:15 P.M.	158
		12:20 P.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		1:15 P.M.	106
		2:15 P.M.	65
		3:15 P.M.	66
4/ 3/35	No. 7	10:45 A.M.	286
		10:50 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		11:45 A.M.	112
		12:45 P.M.	67
		1:45 P.M.	73

through the mucous membrane. Finding several solutions which apparently had this effect, we chose ethylene glycol as a medium. We have employed solutions containing equal quantities of ethylene glycol and insulin, and have also made solutions of powdered insulin in ethylene glycol. The solutions employed for instillation contained 500 units per c.c. With such a solution 0.1 c.c. contains 50 units and 0.2 c.c. 100 units of insulin.

Table I shows a few typical protocols on animals.

CONCLUSIONS

Table I, which is an example of a larger group of similar observations, shows that insulin in ethylene glycol when either dropped or sprayed into the nasal mucous membrane produces an unquestioned and marked fall in blood sugar in normal rabbits, normal dogs, and in diabetic patients. The dosage employed by this intranasal method is considerably greater than that necessary in subcutaneous injection. The fact that insulin under certain conditions can be absorbed from mucous membranes is possibly of more than academic interest.

We are under obligations to Eli Lilly and Company for the insulin used in these observations.

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FUNGICIDES*

I. Influence of Hydrogen Ion Concentration on the Growth of Yeastlike Organisms

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SINCE vulvovaginal mycoses often did not respond well to infrequent local applications of gentian violet and to sodium bicarbonate douches,[6, 7] it was deemed advisable to evaluate the therapeutic efficacy of other agents. Such a problem involved the investigation of numerous compounds, many of which were acid or alkaline or required an acid or alkaline vehicle for solubility or stability. The effects of various hydrogen ion concentrations on the growth of certain test fungi were studied so that this factor might be recognized in subsequent work with antiseptic solutions. Nine strains of pathogenic fungi representative of those isolated from human patients, and a known nonpathogen, *Saccharomyces cerevisiae*,[7] were employed. The hydrogen ion concentrations varied from pH 2.5 to 9.5, since these limits were well beyond those present in physiologic or pathologic vaginal discharges,[1, 4, 5, 8, 10] or therapeutically practicable.

TECHNIC

Two types of media were employed: (a) 5 per cent glucose solution and (b) 0.5 per cent glucose in broth (veal infusion—peptone), each containing sodium monobasic phosphate as a buffer. Although the sodium monobasic phosphate was recognized to be scarcely adequate for these extreme limits, it was chosen so that any possible fungicidal effect would be constant throughout the study. The original medium was made up to pH 5.5, since fungi grew well at this level on dextrose peptone agar slants and in glucose broth. Several volumes of 75 c.c. of medium were adjusted to the various selected hydrogen ion concentrations by the addition of solutions of N/2 NaOH or N/5 HCl, after which 10 c.c. amounts were distributed into culture tubes for subsequent inoculation. By the colorimetric spot-plating method the approximate values of pH 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5 were determined,[2] and the medium was re-checked by the same method after the growth of the fungi. The color indicators were prepared according to standard routines[2] for thymol blue, bromphenol blue, methyl red, bromthymol blue, and cresol red, and were used as prescribed by Clark.[2] Even though this method indicated only approximate pH values, it was sufficiently accurate for this study.

Ten strains of organisms representing three different genera were used. These were saccharomyces, cryptococci and monilia.[3, 9] In view of the con-

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Received for publication, May 21, 1935.
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fusion which existed in mycotic taxonomy, certain cultural characteristics are indicated in Table I. Strain F40 (one of the bakers' yeasts) was a proved nonpathogen,[7] while the remaining cultures were obtained from patients with vaginal or oral mycoses.

TABLE I*

	DEXTROSE	LEVULOSE	MANNOSE	MALTOSE	GALACTOSE	SACCHAROSE	LACTOSE	MILK	MYCELIA	ASCOSPORES	CLASSIFICATION ACCORDING TO MORPHOLOGY	CLASSIFICATION ACCORDING TO FERMENTATION
Saccharomyces												
F40	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	P	Cerevisiae	Cerevisiae
149	⊕	⊕	⊕	⊕	⊕	⊕	−	−	−	P	?	?
Cryptococcus (Torula)												
119	⊕	⊕	⊕	−	−	−	−	−	−	−	?	?
Monilia											Benham	Stovall
104	⊕	⊕	⊕	⊕	⊕	+	−	C	P	−	Albicans	Type 2
120	⊕	+	⊕	⊕	+	+	−	C	P	−	Albicans	Type 2
147	⊕	⊕	⊕	⊕	+	−	−	−	P	−	?	Type 1
152	⊕	⊕	⊕	⊕	−	⊕	−	−	P	−	Candida	Type 3
155	⊕	⊕	⊕	−	+	⊕	−	C	P	−	?	Type ? (variable)
156	⊕	⊕	⊕	⊕	⊕	⊕	−	C	P	−	Albicans	Type 2
157	⊕	⊕	⊕	⊕	+	−	−	−	P	−	Parapsilosis	Type 1

*+ = acid ⊕ = gas − = negative C = coagulation P = produced ? = undetermined

Subcultures were grown on Sabouraud's agar slants and incubated at 37° C. for twenty-four hours. A small loopful of the growth was then transferred to sterile 0.9 per cent NaCl solution in sufficient quantities to make a suspension containing either 50,000 or 500,000 cells in 0.1 c.c. The actual cell counts were made with a standard hematimeter. One-tenth cubic centimeter of the suspension was transferred immediately to each of the 10 c.c. of medium, previously adjusted to the various pH levels, as shown in the tables, and incubated at 37° C. for from eighteen to twenty-four hours, after which one-fiftieth (0.02) cubic centimeters of the suspension of each of the thoroughly agitated cultures was transferred to a Petri dish by using finely drawn pipettes. About 15 c.c. of Sabouraud's medium were then poured into the Petri dish and the culture was mixed by gentle manipulation. Colony counts were made without the use of a magnifying lens following incubation at 37° C. for from twenty-four to one hundred and twenty hours.

RESULTS

Glucose Medium.—To determine the rate of cell reproduction in 5 per cent glucose (Table II and Chart 1), an inoculation of approximately 2,000 yeast cells per 0.02 c.c. of medium was made in each of the original and duplicate tubes for Strains 119, 152, 155, and 156. From actual cell counts, as shown by the circles on the graph, it is evident that many yeast cells died at the low hydro-

gen ion level, while some died even at the supposed optimum level of pH 5.5. The horizontal line represents the number of cells transferred from the original suspension. In general, the rate of growth increased progressively as the acidity approached pH 5.5 from either extreme. In culture No. 152 duplicate, at pH 5.5, the decrease may have been the result of an incomplete suspension of cells before transplantation or insufficiently cooled Sabouraud's medium.

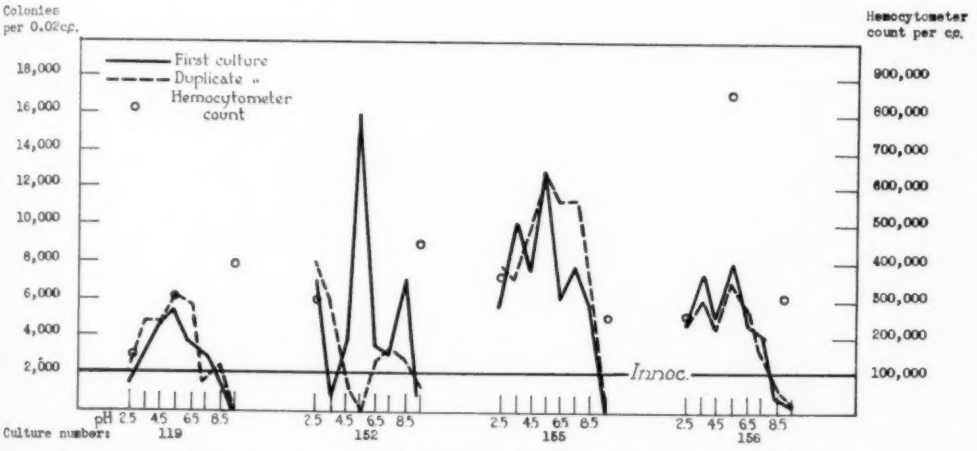

Chart 1.—Hemocytometer and poured-plate counts after forty-eight hours' incubation. Medium 5 per cent glucose.

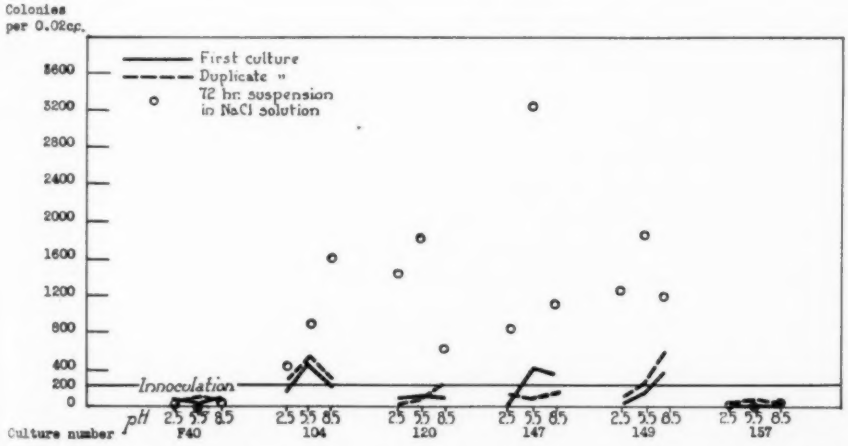

Chart 2.—Poured plate counts from fresh and seventy-two hours' 0.90 per cent NaCl suspensions. Medium 5 per cent glucose.

The remaining six strains (F-40, 104, 120, 147, 149, and 157) were inoculated in duplicate into 5 per cent glucose of pH 2.5, 5.5, and 8.5, as these seemed to be more significant in the earlier tests. The number of cells for each tube inoculation was decreased to 200 per 0.02 c.c. to facilitate the colony count (Table III and Chart 2). An attempt was made in this group to learn whether dilution in 0.90 per cent NaCl solution damaged the cells seriously. After allowing these diluted cultures to stand for seventy-two hours at a

temperature of 22° C. to 37° C., mostly the latter, F-40 and 157 grew poorly, while the others showed better growths in the twenty-four-hour subcultures. This may mean that more cells were inoculated (growth in aqueous NaCl solution), or that the living cells reproduced more rapidly following prolonged exposure to this inadequate medium.

TABLE II

ACTUAL CELL COUNT (HEMATOCYTOMETER) OF TWENTY-FOUR-HOUR 5 PER CENT GLUCOSE CULTURES. ORIGINAL INOCULATION APPROXIMATELY 100 CELLS PER CM.

pH	119	152	155	156
2.5	150	300	350	250
5.5	300			850
9.5	500	450	250	300

COLONY COUNTS AFTER ONE HUNDRED AND TWENTY HOURS IN POURED PLATES (INOCULATIONS FROM TWENTY-FOUR-HOUR 5 PER CENT GLUCOSE CULTURE). EACH 0.02 C.C. HAD 2,000 CELLS WITH ORIGINAL INOCULATION. AVERAGE OF DUPLICATE PLATES

pH	119	152	155	156
2.5	1,883	7,520	6,592	4,750
3.5	4,816	2,960	8,600	6,665
4.5	4,800	2,848	8,932	4,575
5.5	5,787	8,039	12,640	7,424
6.5	4,868	3,010	8,499	4,880
7.5	2,400	3,275	9,480	3,520
8.5	2,080	5,023	5,142	763
9.5	2	848	40	153

TABLE III

COLONY COUNTS AFTER FORTY-EIGHT HOURS IN FOURED PLATES. EACH 0.02 C.C. HAD 200 CELLS WITH ORIGINAL INOCULATION INTO DILUENT. FIRST READINGS ARE AVERAGES OF DUPLICATE TUBES, INOCULATED IMMEDIATELY AFTER DILUTION. SECOND READINGS ARE COLONY COUNTS AFTER FORTY-EIGHT HOURS IN POURED PLATES, INOCULATION MADE FROM DILUENT AFTER IT STOOD WITH CELLS FOR SEVENTY-TWO HOURS

pH	F40	104	120	147	149	157
2.5	5	171	37	66	51	0
	0	420	1,400	800	1,248	0
5.5	12	500	58	272	193	2
	0	900	1,760	3,200	1,856	30
8.5	29	243	168	262	510	0
	13	1,600	668	1,040	1,200	0

Glucose Broth Medium.—Since the fungi grew poorly in a protein-free medium (which is in accord with mycologic reports), 0.5 per cent glucose in broth was employed, using the same technic, except that the number of cells

TABLE IV*

COLONY COUNT AFTER TWENTY-FOUR HOURS IN POURED PLATES (INOCULATIONS FROM TWENTY-TWO-HOUR GLUCOSE BROTH CULTURES). AVERAGE OF DUPLICATE TUBES. EACH 0.02 C.C. HAD 200 CELLS IN ORIGINAL INOCULATION

pH	F40	104	119	120	147	149	152	155	156	157
2.5	5,570	406	436	∝	821	∝	628	12	424	∝
5.5	∝	∝	∝	∝	∝	∝	∝	∝	∝	∝
7.5	–	∝	3,904	–	∝	–	∝	2,745	∝	–
8.5	72	∝	1.384	∝	∝	∝	∝	2,170	2,500	1,700

*– = not run ∝ = innumerable

transferred was reduced from 2,000 to 200 per 0.02 c.c. of the inoculated medium. Strains F40, 120, 149, and 157 were observed in single cultures at pH 2.5, 5.5, and 8.5, while 104, 119, 147, 152, 155, and 156 were run at pH

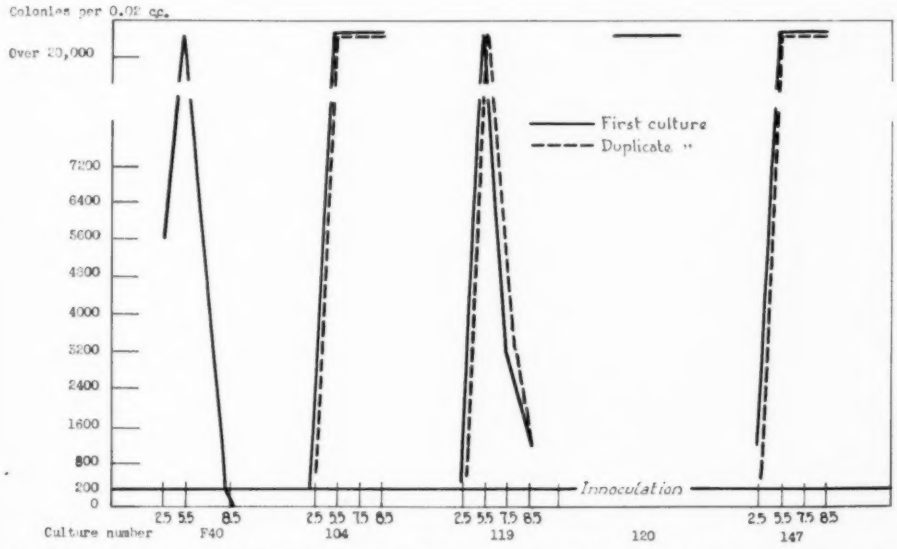

Chart 3.—Colony count after twenty-four hours in poured plates. Medium glucose broth.

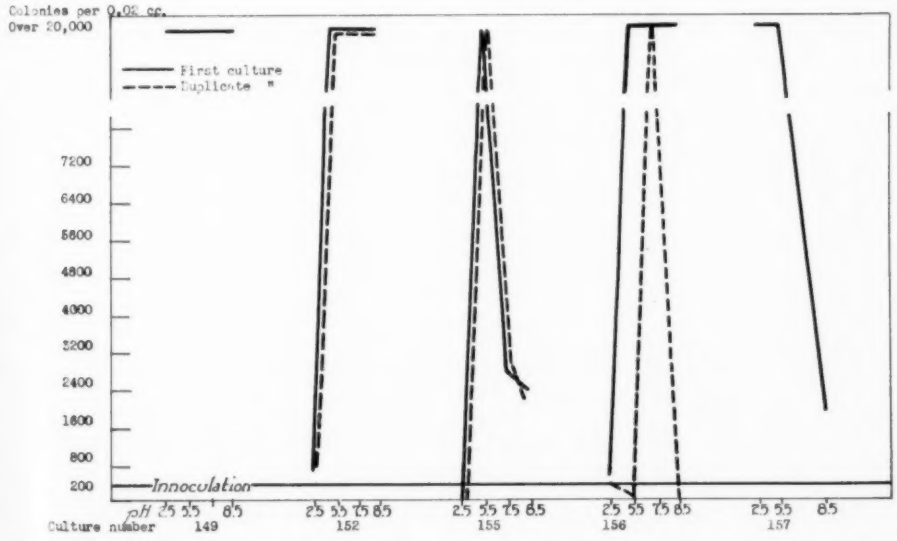

Chart 4.—Colony count after twenty-four hours in poured plates. Medium glucose broth.

2.5, 5.5, 7.5, and 8.5 in duplicate (Table IV and Charts 3 and 4, respectively). The growth was favorable for all strains at pH 5.5, while at the extreme ranges it was variable.

According to Tables II, III, IV and V, and Charts 1, 2, 3, and 4, the pH range from 2.5 to 8.5 represents the outside limits within which appreciable

cell growth occurred in these two media, even for the most versatile yeasts, while pH 5.5 was associated with uniformly good growth.

After cultivation of the inoculated media, the alkaline cultures became more acid, but the pH never changed more than 1.0. With media below pH 5.5 there was no consistent change. Tables V and VI show the time interval and the medium employed in the various tests, and are, perhaps, more significant than a detailed written explanation. In general, the optimum growth is less variable than Talice[11] reports. He states that the optimum pH growth range for cryptococcus is 3 to 9, monilia albicans 7 and saccharomyces 3 to 4.

TABLE V

pH READINGS AFTER TWENTY-FOUR HOURS' CULTURE GROWTH IN 5 PER CENT GLUCOSE. AVERAGES OF DUPLICATE TUBES. SECOND READINGS ARE SEVENTY-TWO-HOUR READINGS

ORIGINAL pH	HOURS	F40	104	119	120	147	149	152	155	156	157
2.5	24	2.5	2.5	3.0	2.5	2.5	2.5	3.0	2.5	2.8	2.5
	72	2.5	2.5		2.5	2.5	2.5				2.5
3.5	24			4.0				4.0	4.0	4.0	
4.5	24			4.5				4.5	4.5	4.5	
5.5	24			5.5				5.5	5.5	5.4	
6.5	24			6.5				6.3	6.5	6.3	
7.5	24			6.6				6.7	6.7	6.7	
8.5	24	8.5	8.5	7.9	8.5	8.5	8.2	7.8	7.8	7.8	8.5
	72	8.2	8.2		8.0	8.2	8.2				8.2
9.5	24			9.2				9.2	9.2	9.3	

TABLE VI

pH READINGS IN GLUCOSE BROTH AFTER FORTY-EIGHT AND SEVENTY-TWO HOURS' GROWTH, AVERAGE OF DUPLICATE TUBES

ORIGINAL pH	HOURS	F40	104	119	120	147	149	152	155	156	157
2.5	48	2.8			2.8		2.8				2.6
	72		2.4	2.3		2.5		2.5	2.5	2.7	
5.5	72		5.6	5.5		5.4		5.5	5.2	5.5	
7.5	72		7.3	7.1		7.1		7.0	7.0	7.1	
8.5	48	7.4			7.8		8.0				7.8
	72		7.8	7.7		7.7		7.5	7.2	8.0	

SUMMARY AND CONCLUSIONS

1. The mycotic organisms studied grew, generally, over ranges greater than are present in the human vagina.

2. One-half per cent glucose in broth was a better medium for the growth of these yeasts than 5.0 per cent protein-free glucose.

3. A pH 5.5 was preferable, but an absolute maintenance at this level was unnecessary for substantial growth of the fungi.

4. Strains 104, 120, 147, 149, and 153 grew well in pH ranging from 7.0 to 7.5, and will permit the study of the fungicidal effects of compounds insoluble, incompatible, or unstable in an acid medium.

5. Fungistatic action may be anticipated usually in some degree with a pH below 4.0 or above 7.5, while fungicidal action was evident at greater extremes.

6. It is suggested that the pathogenicity of a fungous strain may be evaluated by its ability to grow over greater pH ranges. This would suggest that 120 and 149 are more, 104, 147, and 152 less, and 119, 155, and 157 the least pathogenic. This idea is supported partially by the behavior of F-40, a proved nonpathogen.

7. It appears that either alkaline or acid instillations may be inadequate therapeutically because the extreme changes which they induce are only temporary.

8. Further research, which is in progress, concerns the general problem of the fungicidal potency of various agents.

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FUNGICIDES*

II. In Vitro Tests With a Number of Chemicals on Yeastlike Organisms and Other Fungi

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THE clinical importance of infection by the pathogenic fungi has resulted in the publication of a large number of papers dealing with the etiologic agents, structures involved, and the therapy of such infections. The medicaments recommended for the treatment of vulvovaginal mycoses have been tested for ability to kill these organisms in only relatively few instances. The failure of gentian violet and alkaline douches[1, 2] to bring about consistent and satisfactory results in vulvovaginal mycoses when patients cannot return for frequent treatments has stimulated a search for more effective agents.

The present investigation is concerned principally with the group of yeastlike organisms, the Monilias. A review of the work done previously in testing fungicides against the dermatophytes will be presented because of the possible application of the fungicides reported in this paper in the therapy of infections by dermatophytes.

Kolmer and Schamberg[3] tested the fungistatic and fungicidal powers of a number of chemicals against *Trichophyton rosaceum*, *Microsporon audouini* and *Achorion schoenleini*. Iodine, mercuric chloride, and mercurophen were found to be the most effective fungistatic or fungicidal agents.

Kingery and Adkisson[4] found an exposure for seventy-two hours to thymol, 1:6,250, iodine, 1:1,000, benzoic acid, 1:1,000, and salicylic acid 1:1,000, to kill all of several epidermophytons, trichophytons, achorions, a microsporon, a sporotrichum, and the organisms from two cases of blastomycosis.

Kadisch[5] smeared cultures of *Achorion gypseum*, *Trichophyton gypseum*, *Trichophyton depressum*, *Trichophyton acuminatum*, *Epidermophyton lanoroseum*, some thrush fungi, and a cryptococcus on linen cloth which was put in a 1 per cent solution of thymol in alcohol. The dermatophytes were destroyed by a five-minute exposure, but the yeastlike organisms survived for several hours.

Klarmann, Shternov, and Gates[6-8] report germicidal and fungicidal tests with a large number of chlorophenol derivatives. A number of the compounds tried were found effective in dilutions of 1:10,000 to 1:60,000 against *Trichophyton rosaceum*, *Achorion schoenleini*, and *Monilia albicans* in ten minutes.

Yi[9] used *Epidermophyton rubrum*, *Epidermophyton interdigitale*, and *Trichophyton pedis* A and B as test organisms. He reported that these organisms

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Received for publication, May 21, 1935.

This work was supported in part by a grant from Standard Brands, Inc. Ruth Field Curry, B.S., gave special assistance in the early part of this work.

were killed in five minutes by Castellani's fuchsin dye, 1 per cent thymol in 50 per cent alcohol, 0.5 or 1.0 per cent benzoic acid, and 0.5 per cent salicylic acid in 50 per cent alcohol.

Emmons[10] reports high phenol coefficients for sodium hypochlorite or iodine in alcohol or potassium iodide solution on *Trichophyton gypseum* and *Monilia albicans*. A number of chemicals, as mercuric chloride, phenyl mercuric nitrate, formaldehyde, malachite green, gentian violet, sodium ethyl mercurithiosalicylate, hexylresorcinol, thymol, salicylic acid, benzoic acid, and others gave low phenol coefficients.

McCrea[11] found that high dilutions of brilliant green and malachite green were lethal to *Trichophyton interdigitale* and *Epidermophyton rubrum* in one-minute exposure.

Woodward, Kingery, and Williams[12] made tests on cephalosporium, sporotrichum, and *Monilia tropicalis*. An exposure of thirty minutes killed cephalosporium and sporotrichum in high dilutions of n-hexyl resorcinol, n-hexyl ether of resorcinol, chlorothymol, and iodine. These and two additional compounds, 3,5-dibutyl phenol and mercuric chloride, were also highly effective against *Monilia tropicalis*.

EXPERIMENTATION

Many compounds which have been used in vaginal therapy or recommended for the treatment of mycoses were tested under the conditions suitable for fungicidal tests and then under conditions more closely simulating those encountered in clinical use.

Methods.—The strain used in the selection of effective compounds for further tests was a *Monilia albicans* (our Strain 94) which was isolated from a vulvovaginal mycosis. A 0.5 per cent glucose veal infusion broth was inoculated from an active growth on a Sabouraud's glucose agar slant. After eighteen to twenty-eight hours' incubation at 37° C., the cells were suspended in broth by shaking, and equal volumes of the suspension added to solutions of the chemicals in sterile test tubes. These tubes were shaken, and five minutes after mixing (left at room temperature), one 3 mm. loopful of the mixture was transferred to sterile Petri dishes into which Sabouraud's agar was poured at once, and the plates rotated in an attempt to obtain an even distribution of colonies. This method is essentially the same as the transfer method used by Ruehle and Brewer[13] and McCrea.[14] The plates were observed for several days, unless growth occurred earlier. Growth was recorded in terms comparable to that of the control: 4+ represents the maximum, while 0 indicates no growth. The chemicals were used in concentrations which could be employed in vulvovaginal therapy. Anticipating the use of several acids in tests for fungicidal action, the effect of pH was evaluated in preliminary work.[15] It was noted that even after twenty-four hours' exposure at various pH levels, growth occurred at pH ranges of 3.5 to 8.5.

The preliminary test with *Monilia albicans* (Strain 94) served to indicate those reagents which appeared sufficiently promising for further study. These chemicals were then tested in the presence of human serum containing some

red blood cells. No anticoagulants were used in the preparation of the blood serum. The cell counts of these serums varied from 100,000 to 500,000 per c.mm. The serum was mixed with the solution of the chemical a few minutes before addition of the culture suspension. In most experiments the following volumes were used: solution of the chemical 0.5 c.c., serum 0.5 c.c., culture suspension 0.2 c.c. These components were mixed, and transfers made after five minutes as before. The chemicals which proved effective in these tests were then tried against several yeastlike organisms: a cryptococcus and four monilias isolated from vulvovaginitis cases, endomyces* isolated from sputum, and *Saccharomyces cerevisiae*.

Besides the tests on the yeastlike organisms, a number of chemicals were tried against three other fungi: a plant pathogen, *Fusarium nivale*;† a dermatophyte, epidermophyton sp.;‡ and the common salmon pink, fluffy laboratory contaminant, monilia (in the botanical usage). The cultures were grown on Sabouraud's glucose agar, and the growth suspended in saline. These suspensions were added to the solution of the chemical and treated as stated in the tests on Strain 94.

Results.—The results are given in Table I for all compounds tested against *Monilia albicans* (Strain 94). Relatively few chemicals proved effective even under these conditions.

The effective chemicals were then tried against Strain 94 in the presence of serum and cells. The various vehicles which were necessary to obtain solutions of the concentrations used are given in abbreviated form in Table II. Except alcohol, acetone, and water mixture which had little effect these vehicles were not fungicidal. Furthermore, serum and red cells did not inhibit the growth of the cultures as shown in controls. Under these conditions only five of the twenty-four substances killed all of the organisms present, while ten had little fungicidal activity.

Mercuric chloride, mercurophen, phenyl mercuric nitrate, chlorothymol, and iodine element preparations are the five chemicals which seemed to have therapeutic possibilities. The effectiveness of these five chemicals against several yeastlike organisms was next determined. The results are given in Table III. F 40 is a strain of *Saccharomyces cerevisiae*, 119 a cryptococcus, 104, 120, 155, and 156 were monilias, and 56924 an endomyces. With this group of organisms, chlorothymol and mercuric chloride were effective killing agents in a few instances, and yet had very little or no effect in others. Mercurophen failed to kill Strains 104 and 56924. Diluted Lugol's solution, colloidal, or "Safety Iodine," and phenyl mercuric nitrate killed all organisms.

The comparative resistance of four different fungi is given in Table IV. It is to be noted that these tests were not made with serum and cells present. Even under such favorable conditions for fungicidal action, only the colloidal,

NOTE: We wish to express our indebtedness to the following for cultures:

*Dr. W. D. Stovall, Wisconsin Laboratory of Hygiene for the Endomyces culture number 56924.

†Dr. G. K. K. Link, the Department of Botany of the University of Chicago for the *Fusarium nivale*.

‡Dr. Max Obermayer, the Department of Dermatology of the University of Chicago for the epidermophyton sp.

TABLE I

COMPOUNDS TESTED AGAINST *Monilia Albicans* (STRAIN 94) IN BROTH
Exposure Time Five Minutes. ++++ Growth = to Control

	FINAL CONCENTRATION PER CENT	GROWTH		FINAL CONCENTRATION PER CENT	GROWTH
		Acids			
1. Benzoic acid†	0.5	0	7. Pyrogallic acid	0.15	+++
2. Boric acid	2.0	+++	8. Pyroligneous acid	2.5	+++
3. Iodoacetic acid	0.5	++++	9. Salicylic acid	0.5	0
4. Lactic acid	5.0	++++	10. Sulfosalicylic acid	0.25	++
5. Perchloric acid	0.05	++	11. Trichloracetic acid	0.05	++++
6. Picric acid	0.5	++++			
		Elements and Inorganic Compounds			
12. Aluminum sulphate	2.0	+++	20. Potassium iodide	5.0	++++
2. Boric acid	2.0	+++	21. Potassium permanganate	0.05	++++
13. Copper sulphate	2.0	+++	22. Silver nitrate	0.5	++++
14. Gold chloride	0.05	++++	23. Sodium bicarbonate	20.0	++++
15. Iodine			24. Sodium borate	5.0	+++
Tincture iodine	0.5	0	25. Sodium fluoride	0.5	+++
Lugol's solution	0.5	0	26. Sodium hypobromite	0.14	+++
Glycerin solution	0.5	0	27. Sodium hypochlorite	0.1	0
"Safety iodine"	2.0	0	28. Sodium hypoiodite	0.5	+++
16. Lead acetate	0.5	+++	29. Sodium perborate	0.5	++
17. Mercuric chloride	0.05	0	30. Sodium salicylate	2.0	++++
18. Mercuric iodide	0.05	0	31. Sodium thiosulphate*	4.17	++++
5. Perchloric acid	0.05	++	32. Stannous chloride	2.0	+++
19. Potassium chlorate	0.25	++++	33. Zinc sulphate	2.0	++++
		Halogens and Halogen Compounds			
34. Chiniofon*	1.66	+++	18. Mercuric iodide	0.05	0
35. Chloramine	0.5	0	19. Potassium chlorate	0.25	++++
36. Chlorisothymol	0.1	0	20. Potassium iodide	5.0	++++
37. Chlorothymol	0.1	0	39. Sodium chloro-orthophenyl-phenate (Dowicide C)	0.05	0
38. Dettol*	2.08	++			
15. Iodine			25. Sodium fluoride	0.5	+++
Tincture iodine	0.5	0	26. Sodium hypobromite	0.14	+++
Lugol's solution	0.5	0	27. Sodium hypochlorite	0.1	0
Glycerin solution	0.5	0	28. Sodium hypoiodite	0.5	+++
"Safety Iodine"	2.0	0	40. Sodium tetrachlorphenate (Dowicide F)	0.05	+
17. Mercuric chloride	0.05	0			
		Mercury, Silver, and Arsenic Compounds			
41. Argyrol	5.0	++	45. Neoarsphenamine	1.0	++++
17. Mercuric chloride	0.05	0	46. Neosilvol	5.0	++++
18. Mercuric iodide	0.05	0	47. Phenyl mercuric chloride	0.05	+++
42. Mercurochrome	5.0	+++	48. Phenyl mercuric nitrate*	0.04	0
43. Mercurophen	0.05	0	22. Silver nitrate	0.5	++++
44. Merthiolate	0.05	+++	49. Stovarsol (Acetarsone)	2.5	++++
		Dyes			
50. Acriflavin	0.5	+++	42. Mercurochrome	5.0	+++
51. Brilliant green	0.5	+++	43. Mercurophen	0.05	0
52. Crystal violet	0.5	0	55. Metaphen	0.25	0
53. Gentian violet	0.5	++++	56. Methylene blue	0.5	+++
54. Mallophene	2.0	++	57. Pyridium	0.5	++
		Miscellaneous Organic Compounds			
58. Acetaldehyde	0.75	++++	64. Phenol	0.5	++++
59. Aurium ammonium succinimide	0.5	++++	65. Potassium antimony tartrate	0.5	++++
60. Cresol	0.5	0	66. Quinine dihydrochloride	5.0	++++
61. Formal	0.05	+++	67. Resorcinol	1.0	++++
62. Furfuraldehyde	2.0	++++	68. Thymol	2.5	0
63. Hexylresorcinol	0.05	0	69. Urotropin	0.5	++++

*Tested with serum and cells present.

†Compounds are numbered in order of their first appearance in the table.

TABLE II

TESTS WITH *Monilia Albicans* (STRAIN 94) IN BROTH WITH HUMAN SERUM AND RED CELLS ADDED

Exposure Time Five Minutes. ++++ Growth = to Control

CHEMICAL	*VEHICLE	CLINICAL CONCENTRATION PER CENT	FINAL CONCENTRATION PER CENT	GROWTH AFTER EXPOSURE 5 MIN.
1. Benzoic acid	AGW	0.5	0.2	++
9. Salicylic acid	AW	1.0	0.4	+
14. Gold chloride	W	0.1	0.04	++++
15. Iodine				
Lugol's solution	WKI	1.25	0.5	0
Tincture iodine	AW	1.75	0.7	0
	AW	1.0	0.4	+
"Safety Iodine"	W	4.0	1.6	0
	W	1.0	0.4	+++
Glycerin solution	G	1.0	0.4	+++
17. Mercuric chloride	W	0.1	0.04	0
18. Mercuric iodide	WKI	0.1	0.04	+
27. Sodium hypochlorite	W	0.24	0.1	+
31. Sodium thiosulphate	W	10.0	4.17	++++
34. Chiniofon	W	4.0	1.67	+++
35. Chloramine	W	1.0	0.4	+++
36. Chlorisothymol	W	0.5	0.2	++
37. Chlorothymol	AW	1.0	0.4	0
	AGW	0.5	0.2	0
	AGW	0.2	0.08	+++
38. Dettol	W	5.0	2.08	++
39. Sodium chlor-orthophenylphenate (Dowicide C)	W	0.24	0.1	+
40. Sodium tetrachlorphenate (Dowicide F)	W	0.24	0.1	++++
43. Mercurophen	W	0.2	0.08	0
	W	0.1	0.04	+++
47. Phenyl mercuric chloride	Walk.	0.1	0.04	++
48. Phenyl mercuric nitrate	AWalk.	0.1	0.04	0
49. Stovarsol (Acetarsone)	W	5.0	2.0	+++
	W	2.0	0.8	++++
52. Crystal violet	W	1.0	0.4	+
55. Metaphen, tincture	A Ac W	0.2	0.08	+++
	AW	0.13	0.05	++++
60. Cresol	W	1.0	0.4	+++
63. Hexylresorcinol	W	0.1	0.04	+++
68. Thymol	AGW	0.25	0.1	+++

*alk., Alkali
A, Alcohol
Ac, Acetone
G, Glycerin
KI, Potassium Iodide
W, Water

TABLE III

TESTS WITH A NUMBER OF YEASTLIKE ORGANISMS IN BROTH WITH HUMAN SERUM AND RED CELLS ADDED

Exposure Time Five Minutes. ++++ Growth = to Control

	FINAL CONCENTRATION PER CENT	ORGANISMS TESTED						
		F40	140	119	120	155	156	56924
Chlorothymol	0.21	0	+++	++	+	0	++	0
Iodine								
Lugol's solution	1.03	0	0	0	0	0	0	0
"Safety Iodine"	1.64	Not run	0	0	0	0	0	0
Mercuric chloride	0.04	+	+++	++++	++	0	++	++++
Mercurophen	0.08	Not run	++	0	0	0	0	+++
Phenyl mercuric nitrate	0.04	0	0	0	0	0	0	0

or "Safety Iodine" and sodium chlor-orthophenylphenate (Dowicide C) killed all four fungi. There was considerable difference in resistance among the four types of fungi, but in general, *Monilia albicans*, Strain 94, was the more resistant.

TABLE IV

COMPARATIVE SUSCEPTIBILITY OF FOUR FUNGI TO VARIOUS CHEMICALS
Exposure Time Five Minutes. ++++ Growth = to Control

CHEMICAL	FINAL CONCENTRATION PER CENT	*Monilia Albicans* STRAIN 94	*Fusarium Nivale*	MONILIA SP. (SITOPHILA TYPE)	EPIDERMOPHYTON SP.
Benzoic acid	0.5	0	0	0	+
Formaldehyde	0.15	+++	0	++	++
Furfural	2.0	++++	+	++++	++
Iodine "Safety Iodine"	2.0	0	0	0	0
Iodoacetic acid	0.5	++++	0	+	0
Mercuric chloride	0.05	0	Not run	Not run	+
Mercurophen	0.05	0	0	+++	+
Merthiolate	0.05	++++	0	0	+
Phenol	0.5	++++	++	++++	Not run
Picric acid	0.5	++++	0	0	+
Pyrogallic acid	0.15	+++	++	++++	Not run
Salicylic acid	0.5	0	0	0	+
Sodium chlor-orthophenylphenate (Dowicide C)	0.05	0	0	0	0
Sodium hypochlorite	0.1	0	0	+	0
Sodium tetrachlorphenate (Dowicide F)	0.05	+	++	+	+

The reviewed literature indicates that the following chemicals have fungicidal activity toward dermatophytes and monilias: iodine, mercuric chloride, mercurophen, thymol, n-hexylresorcinol, salicylic acid, benzoic acid, brilliant green, malachite green, and chlorophenol derivatives. These compounds, with the exception of malachite green and the chlorophenol derivatives, were tested under conditions not radically different from those employed by the authors mentioned. Under these conditions, the results in the previous reports were confirmed. Malachite green, brilliant green, but more often crystal violet and gentian violet have been recommended for mycotic infections. In tests performed in broth, only crystal violet killed *Monilia albicans* (Strain 94) in the five-minute exposure time.

In none of the studies reviewed were the organisms tested under conditions approaching in vivo states. In an attempt to approach the in vivo environment, human serum and blood cells were added. Whereas the previous tests would have left the impression that a number of active fungicides have been included in the list of chemicals studied, the test with serum and cells present indicated that only a very few of these chemicals possessed fungicidal properties under conditions simulating those in obstetric and gynecologic therapy. A number of the remaining chemicals would kill organisms if exposed for a longer period of time, however in therapy within body cavities such as the vagina, it is difficult to obtain prolonged contact between the medicament and the entire surface. The chemicals showing the most promise for clinical application are iodine as Lugol's solution (1:2) or a colloidal

preparation ("Safety Iodine") and phenyl mercuric nitrate 1 to 1,000. The 1:2 Lugol's solution has produced irritation in some cases, and in them a 1:4 dilution was used. The "safety iodine" was not irritating when properly suspended and when no deterioration had taken place. The phenyl mercuric nitrate was dissolved in 15 per cent alcohol and 2 per cent 0.1 N sodium hydroxide.

The assumption is not uncommon that a compound which has fungicidal action may be active against fungi in general. From Table IV it may be seen that this view has little support when formaldehyde, a reputedly good fungicide, had very little effect on *Monilia albicans*, Strain 94. In addition to formaldehyde, furfural, merthiolate, and picric acid showed extreme variability in their power for killing the different species. The difficulty in finding a universal fungicide is apparent especially when even the closely related yeastlike organisms show moderate variability in their susceptability to fungicides (see Table III).

CONCLUSIONS

1. Employing a loop transfer method of testing, a considerable number of chemicals were capable of killing *Monilia albicans* (Strain 94) in five minutes exposure in concentrations clinically usable.

2. In the presence of human serum and blood cells, mercuric chloride, merceurophen, phenyl mercuric nitrate, chlorothymol, dilute Lugol's solution, and colloidal iodine killed all organisms of Strain 94.

3. Seven additional organisms, a saccharomyces, a cryptococcus, four monilias, and an endomyces were subjected to the chemicals enumerated in two, under similar experimental conditions. It was noted that only phenyl mercuric nitrate, the diluted Lugol's solution, and the colloidal iodine preparation were effective against the organisms tested.

4. Tests for killing power were made with 15 compounds on 4 unrelated fungi, 2 of medical, and 2 of botanical interest. The results show obvious differences in strain susceptability.

5. Even within a genus, considerable difference may exist in susceptability to fungicides.

6. Before any compound can be asserted to be a fungicide, it must be tested against many varieties of organisms for which it is to be used therapeutically.

7. The existence of an universal fungicide suitable for clinical use is extremely doubtful.

8. The more promising compounds are being tried in clinical studies of vulvovaginal mycoses.

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THORACIC DUCT LYMPH PRESSURE IN CONCRETIO CORDIS*

An Experimental Study

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PICK'S disease or concretio cordis was produced in two dogs by the introduction of aleuronat into the pericardial cavity. This substance is irritating, and it results first in the formation of fluid in the pericardial cavity and later in the fusion of the pericardium and epicardium. The venous pressure

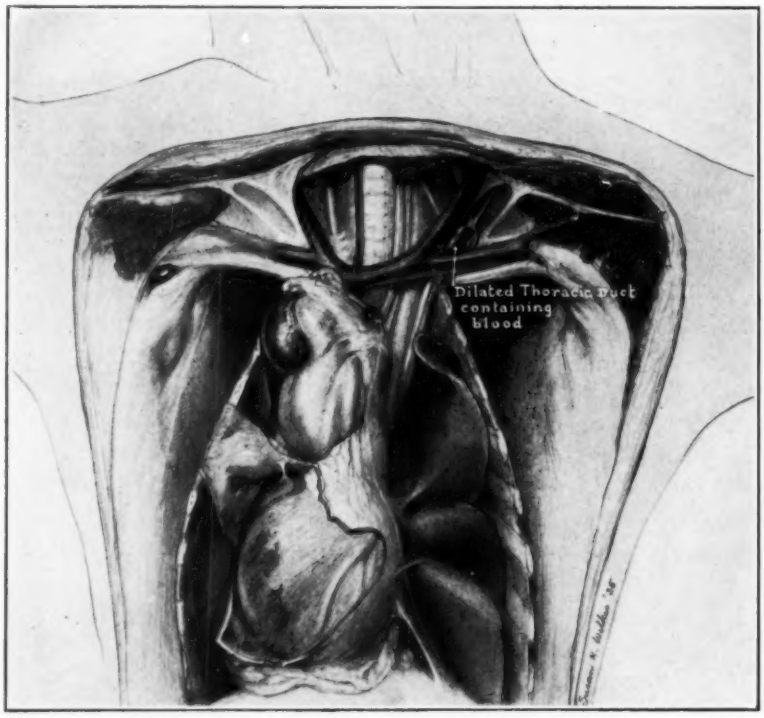

Fig. 1.

rises and fluid accumulates in the peritoneal cavity. Similar changes were produced by Beck and Griswold† by the introduction of Dakin's solution into the pericardial cavity.

Three weeks following the introduction of aleuronat into the first dog, the pressure in the external jugular vein was 120 mm. water and that in the

*From the Departments of Surgery and Medicine of Vanderbilt University.
Received for publication, May 24, 1935.
Aided by a grant to Vanderbilt University from the Division of Medical Sciences of the Rockefeller Foundation.

†Beck, C. S., and Griswold, R. A.: Pericardiectomy in the Treatment of the Pick Syndrome, Arch. Surg. 21: 1064, 1930.

femoral vein was 140 mm. water. Under morphine narcosis, the cerebrospinal fluid pressure was found to be 240 mm. water. Under ether anesthesia, the thoracic duct which was markedly dilated was exposed in the neck. Blood was present in the duct for a distance of approximately 1 cm. peripheral to its entrance into the vein. By inserting a needle into the duct and allowing salt solution to flow in, the pressure in the duct was found to be 150 mm. water while that in the subclavian vein was 165 mm. water. The animal was killed and a typical instance of concretio cordis was demonstrated. Twelve hundred cubic centimeters of fluid were present in the peritoneal cavity and an exudate was found on the surface of the liver. A drawing of the autopsy specimen is shown in Fig. 1.

In the second experiment, the animal appeared ill seventeen days following the introduction of aleuronat. The pressure in the external jugular vein was 155 mm. water and that in the femoral vein was 175 mm. water. The cerebrospinal fluid pressure was 200 mm. water. Using ether anesthesia, a markedly dilated thoracic duct was exposed. A small amount of blood could be seen during expiration in the duct at its entrance into the vein. The pressure in the thoracic duct was found to be 200 mm. water and that in the subclavian vein to be 175 mm. water. Following the removal of the needle from the duct, a pulsating stream of lymph shot out through the needle hole during each expiration. Several hundred cubic centimeters of lymph escaped during the thirty minutes that the duct was exposed. The incision was closed and the animal died unexpectedly two days later. There were 1,100 c.c. of blood tinged fluid in the pleural cavities and 90 c.c. of thin yellowish fluid in the peritoneal cavity. The pericardium and epicardium were fused and thickened. There was an exudate covering the liver.

SUMMARY

Concretio cordis was produced in dogs by the introduction of aleuronat into the pericardial cavity. The thickening and fusion of the pericardium and epicardium were associated with a dilatation of the thoracic duct, with a lymph pressure elevated to approximately the same extent as the venous pressure, and with a small amount of blood in the proximal end of the duct. No conclusions are drawn as to the part if any which the elevated lymph pressure plays in the accumulation of fluid in the serous cavities.

THE DIURETIC ACTION OF MERCUPURIN*

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EVER since the rediscovery of the use of mercury as a diuretic by Jendrassik[1] in 1886, mercury has been an important adjunct in the treatment of cardiac dropsy. Jendrassik, believing a case of dropsy to be of syphilitic origin, used calomel and jalap in the treatment. So effective were the results that further investigation revealed that it was the calomel which was the diuretic agent. The use of mercury in edema was given further impetus by the discovery and introduction of organic mercury compounds in 1917. This time Zieler[2] introduced novasurol as an antisyphilitic. The diuretic effect of this drug, however, was described by Saxl and Heilig[3] in 1920. In 1924 salyrgan was introduced by E. Bernheim[4] as another organic mercury diuretic.

Novasurol, merbaphen, is the double salt of sodium mercuriphenyl oxyacetate with diethylbarbituric acid and contains 33.9 per cent mercury directly united to the benzene ring. Salyrgan, mersalyl, on the other hand is an aromatic compound containing 39.6 per cent mercury in the form of the hydroxy mercuric radical, HgOH, firmly attached to the end of a side chain. Mercupurin, introduced in foreign literature as novurit, is the third and newest of the group of organic mercury diuretics. This compound is the sodium salt of trimethylcyclopentanedicarbonicacid—methoxymercuryallylamide —theophylline.

Following the animal experiments of Issekutz and Vegh[5] it was found that mercupurin was less toxic than novasurol and salyrgan and that it had greater diuretic effects than its predecessors. Clinical tests by Berger, Fazekas and Galgoczy[5] in Hungary confirmed the animal experiments of Issekutz and Vegh.

Further clinical tests by Popper[6] and Hahn[7] agree that mercupurin is an efficacious mercury diuretic without toxicity.

Since there has been no confirmation of these reports in American literature up to the present time, we have undertaken to establish the value of this drug in the treatment of cardiac edema. We have confined our experiments to the use of mercupurin intravenously in 2 c.c. doses. For the most part the patients studied were placed on the usual cardiac routine for several days before the drug was administered. This was particularly true of the earlier cases. The routine consisted of bedrest, digitalis, ammonium chloride, and restricted fluids. When it was noted that the limit of renal output was established, mercupurin was given. The daily output of urine was recorded as well as the daily weight of the patient. We felt that the weight was a

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Received for publication, May 24, 1935.

TABLE I

TABLE I

	NAME	AGE	SEX	DIAGNOSIS	WEIGHT DAY OF INJECTION IN LB.	FIRST 24 HR. URINE IN C.C.	FIRST 24 HR. WEIGHT IN LB.	SECOND 24 HR. URINE IN C.C.	SECOND 24 HR. WEIGHT IN LB.	THIRD 24 HR. URINE IN C.C.	THIRD 24 HR. WEIGHT IN LB.	URINE BEFORE MERCUPURIN ALB.	CASTS	W.B.C.	R.B.C.	URINE AFTER MERCUPURIN ALB.	CASTS	W.B.C.	R.B.C.	MAXIMUM WEIGHT LOSS PER INJECTION (LB.)
1	M. B.	34	m	Mitral stenosis	155	1300	151	2200	156	1200	156½	+++	Occ.	Occ.	Rare	++	Rare	Rare	Rare	4
2	G. E.	71	m	Hypertension	171			2150	155	2100	148	Tr.	None	None	None	None	None	None	None	23
3	N. H.	37	m	Rheumatic	154	2300	149½	1000	150¼		152	+++	Occ.	Occ.	None	None	Occ.	Occ.	None	4½
4	G. W.	60	m	Hypertension	154¾	2000	147	1500	146	1100	144¾	++	Many	None	Rare	None	None	None	None	10
5	E. H.	63	m	Coronary	(1) 163½	2600	154½	1850				++	Occ.	Few	None	++	None	Few	None	
					(2) 158	4600	137	3000	136½	950	136					++	Occ.	Occ.	None	22
6	M. M.	44	f	Aortic insufficiency syphilitic	(1) 245	1360	237	450	236	1250	235	+++	None	None	None	+	None	None	None	10
					(2) 210	4060	208	2400												
					(3) 185	2500	175	1950	176	550	177¼									
					(4) 178	900	173	2150	174	425	175¾									
					(5) 179	1000	177	850	178¼	625						+	Occ.	Few	None	
7	M. C.	55	f	Hypertension	168	2450		1450		900	155	++	Rare	Rare	None	None	None	Rare	Rare	13
8	B. C.	50	f	Hypertension	(1) 164	2570	146½	4150	145	300	147	+	Occ.	Occ.	Rare	++	Many	Many	None	
					(2) 151½	2370	131¼	2350	131	250	133					+++	Many	Few	Few	20½
9	F. M.	50	m	Coronary	156½	3300	146	3900	134	3100	132½	++	None	None	None	None	None	None	None	24
10	E. S.	41	m	Mitral stenosis	182	6300	164	5900	155	1300	157	+	None	Few	None	+	None	Rare	Rare	27
11	J. H.	54	m	Coronary	142	4350	129	3700	123	1500	124	+	None	Few	None	None	None	None	None	19
12	L. M.	60	f	Coronary	(1) 164	4015	151	3210	151	600	151	++++	Occ.	Occ.	Rare	++++	Occ.	Occ.	Occ.	
					(2) 152	250	144	2000		500	146½									
					(3) 160	5150	146	2750	146	1150						++++	Occ.	Occ.	Rare	14
13	F. W.	62	f	Coronary	153	1800	155	2975	151	1150	152	+++	Occ.	Occ.	Rare	++	Rare	Rare	None	3-4
14	C. S.	50	m	Hypertension	168	2850	159	2100	160	1400		++++	None	None	Rare	Lethal exodus				9
15	H. B.	73	m	Coronary	(1) 172	5000	165	2400	165	950	166	+	Rare	None	None	Tr.	Rare	Rare	None	8
					(2) 172	1000	165	900	164		166									
					(3) 167	1500	162	5100	164	950	163					Tr.	Rare	Rare	None	
16	E. G.	49	m	Hypertension	136	2300	129	700		1200	125	++++	Rare	Occ.	None	++	Occ.	None	None	11
17	G. B.	68	m	Hypertension	134	1900	116	1750	112	1400	108	++	None	Occ.	None	Tr.	None	Occ.	None	26
18	O. M.	43	m	Hypertension	(1) 216	4950	202	4000	200	3000	200	Tr.	None	None	None	None	Occ.	Occ.	None	20
					(2) 190	3400	173	1500	170	700	177									
					(3) 207	3500	191	4300		1100	196					Tr.	Many	Occ.	None	
19	A. P.	56	m	Emphysema	208	600	199	1500	192	1100	183	Tr.	None	None	None	+	None	None	None	25
20	A. R.	33	m	T.B. pericarditis	(1) 140	2000	128	4000	128	1450	132	+	None	Rare	None	+	None	None	None	12
					(2) 140	3400	134	1400	131	200						+	None	None	None	
21	F. W.	33	f	Hypertension	148	6350	128	3120	127	620	129	None	None	None	None	+	None	Occ.	None	21
22	J. M.	42	m	Hypertension	163	4600	133	1600	134	760	136	++	None	None	None					30

more accurate index of the fluid loss than the estimate of the urine, as in many instances patients were incontinent.

Twenty-two patients were studied and a total of thirty-five intravenous injections of mercupurin were given. No nephritis, gastro-enteritis or untoward reactions of any kind followed the use of the drug. The following protocol illustrates the type of patient treated and the fluid and weight loss.

SUMMARY

1. Mercupurin was used intravenously in 2 c.c. doses in twenty-two individual cases.

2. Thirty-five injections were administered.

3. Mercupurin proved to be an effective diuretic.

4. No ill results were noted.

5. Weight losses ranging from 3 to 30 pounds per injection were attributable to the diuretic action of the drug.

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LABORATORY METHODS

PREPARATION OF THE KRUEGER UNDENATURED BACTERIAL ANTIGENS*

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INTRODUCTION

In the past, attenuation of various virus antigens by physical or chemical means or by propagation in heterologous species has contributed greatly to the success of protective artificial immunization. On the other hand bacterial pathogens and their products have been subjected to such a variety of conditions and methods of treatment in their preparation for use as antigens that almost all degrees of success of protective artificial immunization have been obtained at some time or other.

For example, Pasteur's chicken cholera vaccine was considerably attenuated in virulence by age and presence of accumulated metabolic substances; however, it was highly immunizing and no doubt much native bacillary substance was present in the form of organisms which remained viable. Heat-treated typhoid vaccine may be mentioned as another immunizing antigen of well-known effectiveness. In this instance, while the vaccine comprises killed microorganisms, it is nevertheless quite useful in human immunization, and may be cited as a strong antigen of much more than average stability against heat. A third antigen, or rather series of antigens, is of interest, namely, that derived from the diphtheria bacillus. In this instance administration of highly antigenic toxin is made practical by modifying it with antitoxin or attenuating it with formaldehyde, and subsequently by rendering the product of this latter treatment rather insoluble to allow of longer tissue stimulation. Units of measurement of antigenicity and corresponding immunity are so well known in this instance, that a high degree of precision is attained in practical immunization. A fourth type of antigen, namely bacteriophage antigen, has been the subject of an enormous amount of work. A useful kind of devitalization of some bacteria may be brought about by the use of bacteriophage, and lysate antigens so prepared may have more merit in some instances than corresponding heat-treated antigens. We do not consider the direct participation of bacteriophage itself, but regard it as a means of producing solutions of bacterial substance.

Increasing interest has been exhibited in the last few years in what actually happens to bacillary substance in preparing bacterial antigens. Perhaps where viable bacteria can be used, or where the antigen, be it heterophile or not, is unusually heat stable, or where the technic of antigen preparation is so exactly reproducible due to ease of determination of units of activity, the prob-

*Presented, in part, at the Thirty-Sixth Annual Meeting of the Society of American Bacteriologists in Chicago, December 28, 1934.
Received for publication, April 4, 1935.

lem is not so urgent. However, many bacteria are left on the list, artifical immunity to which presents much room for improvement and an obvious need for more useful antigens.

The older work of Obermeyer and Pick[1] on denaturation of protein and corresponding immunologic responses, and the more recent contributions to this subject by Anson and Mirsky[2] have centered attention in vaccine preparation upon control of denaturation processes commonly occurring in bacterial antigen preparation.

We have experimented with devitalization of bacteria with the noncoagulating antiseptic merthiolate[3] as an improvement over heat treatment of bacteria in the preparation of more nearly natural or native antigens. More recently Krueger and his associates[4] have described experimental methods for mechanical devitalization and fragmentation of bacterial cells and subsequent merthiolate preservation of the antigen causing practically no denaturation of protein. In this report we wish to describe experimental preparation of the Krueger undenatured bacterial antigen ("U.B.A.") and some laboratory results of assaying different preparations of such bacterial substances.

EXPERIMENTAL

We have prepared, on an increasingly large scale, undenatured bacterial antigens from *H. pertussis*, from several other respiratory bacteria, including pneumococci, streptococci, and *H. influenzae*, from staphylococci, from colon and typhoid bacilli, and from gonococci.

A. Bacterial Culture and Culture Media Utilized.—H. pertussis: freshly isolated strains grown on human or sheep blood (25 per cent) Bordet agar were utilized up to six months of age. All of these cultures were smooth, hemolytic, agglutinated well with "Phase 1" antiserum, and as far as could be determined fulfilled the characteristics of S strains as recently defined by Shibley.[5] Each lot of pertussis U.B.A. was prepared in the following way: Not less than five nor more than twenty-five cultures were utilized as seed in the form of a forty-eight-hour growth on large Bordet blood agar tubes. The growth from each large tube was suspended in 10 c.c. Bordet "potato juice" and planted in 2.5 c.c. amounts to four Bordet blood agar blake bottles. At first a lot comprised twenty-four such blake bottles, but later from a practical standpoint the size of each lot was increased gradually up to four hundred blake bottles. Fig. 1 shows the appearance and method of handling such a lot of bottles. After incubation at 35° C. for forty-eight hours, the pure culture growths were washed from the medium with sterile Locke-Ringer solution.

Pneumococci and Streptococci: The pneumococcus cultures included Types 1, 2, 3, and a member of Cooper's Type 4, and also a well-known broad heterophile antigen strain "DRI" originally coming from the Neufeld Type I strain. The first four cultures were mouse passaged once weekly and kept in rabbit blood beef infusion broth, while the DRI culture was simply cultivated in this medium without passage. The streptococci included at first representative hemolytic, viridans, and indifferent strains used to produce regular bacillary vaccine in this laboratory. Later two highly virulent mouse passaged streptococcus strains were added which regularly killed mice in doses of 10^{-7} or 10^{-8}

c.c. Each lot of pneumococcus or streptococcus U.B.A. was prepared by planting rabbit blood broth seed cultures into lots comprising usually sixteen large three-gallon bottles of freshly made warm beef infusion broth to which 0.1 per cent dextrose was added after sterilization, and growing these at 37° C. for

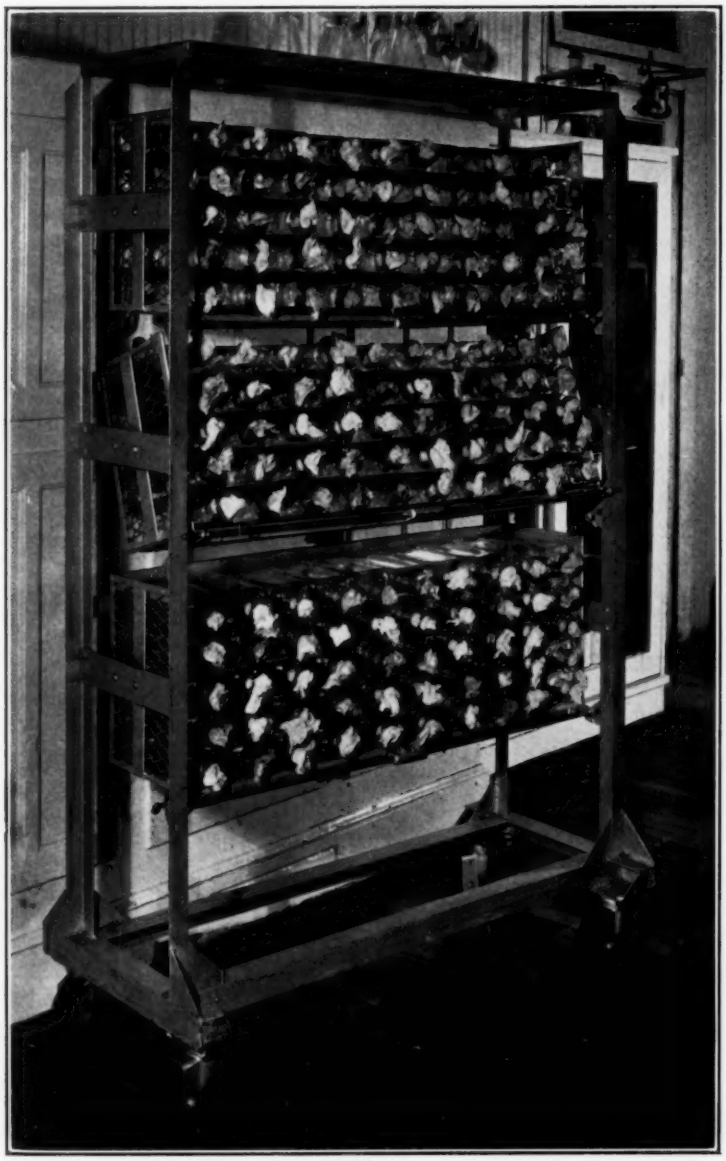

Fig. 1.

twenty-four hours. The bacteria were recovered from these massive cultures through the use of a Sharples centrifuge, and suspended in Locke-Ringer solution.

H. influenza: Four laboratory strains were utilized at the start of the work. Later two smooth strains, representative A and B types supplied by

Dr. Margaret Pittman, were included, and subsequently an Indianapolis strain freshly isolated from a case of pharyngitis, and having definite virulence in pure culture for mice, was added. These organisms were grown twenty-four hours in cleared chocolate broth at 37° C. and the bacteria were recovered with a Sharples centrifuge and suspended in Locke-Ringer solution.

Colon and Typhoid Bacilli: Bacillary vaccine laboratory strains were used, and each lot of undenatured bacterial antigen was prepared by planting broth seed culture to blake bottles of dextrose beef infusion agar and growing these at 37° C. for twenty-four hours. The bacterial growth was washed from the agar and suspended in sterile Locke-Ringer solution.

Gonococci: Bacillary vaccine laboratory strains were grown forty-eight hours on blake bottles of testicular agar as for regular vaccine preparation. The bacterial growth was washed from the agar and suspended in sterile Locke-Ringer solution.

B. *Subsequent Treatment of All Suspensions of Bacteria in Locke-Ringer Solution Immediately on Recovery From Culture Medium.*—These suspensions were rapidly sedimented at high speed on an International centrifuge, the supernatant fluid discarded, an additional Locke-Ringer solution added, and the sediment resuspended. This process of washing was again repeated, and the final bacterial sediment was made up to a thick consistency with additional Locke-Ringer solution.

At this point the washed suspensions were ground according to the Krueger technic in glazed ball mills about three-quarters full of three-eighth-inch stainless steel balls, rotating at speeds which may vary from 30 to 120 revolutions per minute. Grinding was continued for from sixteen to twenty hours at which time nearly all cells were fragmented and very few intact bacterial cells were present as shown by stained smears. The solutions then were spun lightly on an International centrifuge to remove coarse particles and thereupon were ultrafiltered.

Filter membranes were relatively nonadsorptive and were prepared from Whatman number fifty filter papers impregnated in the usual way with a 4.5 per cent solution of acetic cotton. Ultrafilters supporting membranes 7 cm. in diameter, and holding a volume of about 200 c.c., were used at first. Later, larger ultrafilters, supporting membranes of 18.5 cm. in diameter and holding a volume of about 2,000 c.c., were used to accelerate filtration. Fig. 2 shows the appearance of these ultrafilters.

Denaturation of bacillary protein during the grinding procedure was prevented by use of buffered solution, slow speed of the ball mills avoiding friction heat, and conducting the grinding in quarters not subjected to overheating. Denaturation during ultrafiltration was avoided by rapidity of procedure thus preventing prolonged exposure of the protein substrate to viable bacteria which may have escaped grinding, and conducting the ultrafiltration in laboratory quarters also not subjected to overheating. The ultrafiltrates were preserved with merthiolate 1:100,000, instead of the phenoloid preservatives. This concentration was used since it was well beyond the denaturing concentrations of this chemical for native bacillary protein in such pure state.

C. *Standardization of U.B.A. Ultrafiltrates.*—Micro-Kjeldahl nitrogen determinations were made upon each separate lot of the various U.B.A. preparations. These values varied mainly from about 5 mg. to about 75 mg. nitrogen per 100 c.c. of ultrafiltrate depending on the species of microorganism used, and the density of the suspension subjected to grinding. Two U.B.A. (staphylococcus and pneumococcus) lots were made which contained over 100 mg. nitro-

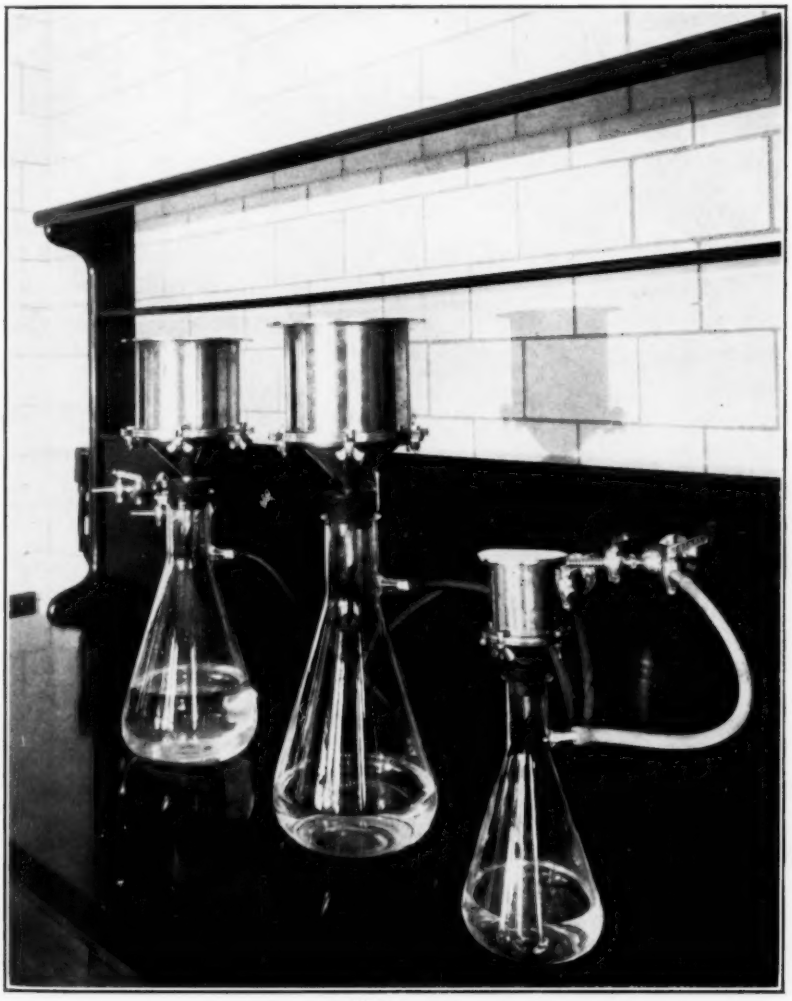

Fig. 2.

gen per 100 c.c., while the average preparations contained about 25 mg. nitrogen per 100 c.c. (see Table I).

At the suggestion of Dr. A. P. Krueger we have tested these U.B.A. ultrafiltrates further for undesirable denatured protein by isoelectric precipitation at about pH 4.5. About 1 per cent of such antigens show traces of denaturation and these have been discarded as unsatisfactory for further experimental use.

Utilizing these two criteria of excellence, preliminary trial showed that human treatment could most satisfactorily be conducted with pertussis U.B.A.

TABLE I

UNDENATURED BACTERIAL ANTIGENS (U. B. A.): LABORATORY LOTS OF VARYING SIZE AND CONCENTRATION

U. B. A.	AMOUNT MEDIA (BLAKE BOTTLES OR LITERS OF BROTH)	PREPARATION NUMBER	VOLUME (CUBIC CENTIMETER)	NITROGEN (MGM.) PER 100 C.C.
Pertussis	100 bottles	B–2947	225	13.48
Pertussis	100 bottles	B–2948	240	10.41
Pertussis	172 bottles	B–2955	170	41.08
Pertussis	150 bottles	B–2958	280	26.64
Pertussis	150 bottles	B–2967	375	14.44
Pertussis	300 bottles	902009	800	16.80
Streptococcus	30 liters	B–2946	100	69.68
Staphylococcus	25 bottles	B–2888	70	141.92
Pneumococcus	160 liters	902812	2500	19.76
Gonococcus	300 bottles	902005	425	19.5

containing 10 mg. nitrogen per 100 c.c., while optimum concentration of nitrogen in "respiratory," staphylococcus, streptococcus, and gonococcus U.B.A. was 5 mg. per 100 c.c. These undenatured bacterial antigens have been supplied to a large number of clinical investigators for evaluation. Some of their results have appeared in the literature, and further reports are in progress. Suffice it to mention here that their use appears definite and readily demonstrable, so far as such studies have progressed and their widest respective clinical use has been in the treatment of pertussis, chronic paranasal sinusitis and other respiratory conditions.

D. *Comparative Properties of U.B.A.*—As compared to conventional vaccine, U.B.A. is quite free of undesirable bacillary metabolic products, and culture media ingredients, and also is relatively atoxic. In terms of numbers of original bacteria represented, a much greater amount of native U.B.A. can be conveniently administered than can with the conventional vaccine. Reactions from several multiples of an optimum human dose comprise slight local stiffness and sensitiveness to pressure. There is an unusual freedom from general systemic reactions of any sort. Theoretically U.B.A. offers a good substitute for living vaccine. It comprises fragmented cells and native bacillary protein solution uncontaminated with so-called nonspecific substances, and unchanged by the technic of preparation. It is a mild antigen as compared with many irritating vaccines. The sum total of immunologic response is not dissipated against associated split products and denatured proteins following the use of U.B.A. Antibody response in animals has been only partly investigated, and although agglutinins and other antibacterial bodies are produced, they are not spectacularly high titered, but in some instances appear somewhat earlier than usual. Rapidity of therapeutic action of pertussis U.B.A. suggests some form of desensitization or altered tissue reactivity as the likely mechanism of action, rather than immunization as commonly thought of. Also, since antibody such as agglutinin does not regularly parallel immunity, either active or passive, the titer of serum antibody following U.B.A. treatment is not an accurate indicator of resistance. It follows that precision laboratory tests of the degree of resistance incited by U.B.A. are yet to be devised.

SUMMARY

By utilizing carefully selected cultures as the starting point, and the application of large scale bacteriologic procedures, we have prepared the Krueger U.B.A. in increasing large quantities for clinical use. The Krueger U.B.A. is quite free of the degradation products associated with conventional vaccine.

These antigens have been standardized to desired nitrogen content when finished, and are preserved with merthiolate. Accidental or unforeseen denaturing effects occurring during preparation are detectable in the final laboratory examination of each antigen. Through elimination of any antigen showing a trace of denaturation as witnessed by isoelectric flocculation of denatured protein, a supply of native bacterial antigen is assured. U.BA. in quantity has been produced mainly from *H. pertussis*, several bacteria associated with upper respiratory disease, pyogenic cocci, and the gonococcus.

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A MORE SENSITIVE COMPLEMENT FIXATION TEST FOR GONORRHEA*

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THE classical complement fixation test for gonorrhea has not given satisfactory results. By the customary gonorrhea fixation test, it is seldom that clear cut positive and negative reactions are obtained.[1] When attempts are made to obtain definite positive results in known cases, a number of indefinite reactions are obtained upon patients who are undoubtedly negative. For these reasons, the test is carried on by few laboratories.

The complement fixation test for gonorrhea presented here is much more sensitive in the determination of immune substances than existing tests. Work with children in institutions indicates that it does not give false positive or indefinite results except in meningitis.

In addition, it is truly quantitative because it determines accurately the amounts of complement absorbed nonspecifically by every specimen. Furthermore, it takes into account that quantitative relations do not exist between reaction tubes which contain varying amounts of serum. In this test, the specific fixation by the patient's serum is read in conjunction with the amount of nonspecific absorption of complement, and the same amounts of serum are used in the control and each of the reaction tubes.

The method presented here requires nothing more than a readjustment of the quantities of reagents used and a new interpretation of readings. The reasons behind the changes are important to complement fixation work in general, and to the gonorrhea fixation in particular.

The question has often been raised as to whether complement fixing bodies were developed in gonorrhea infections in sufficient quantities for practical diagnosis. Because of the fact that it was not always possible to demonstrate their presence in many known cases of gonorrhea, and because the fixation of complement was slight when it did occur, this has been a difficult question to answer.

Complement fixing bodies are present in gonorrheal serums, but they are present in such small amounts that the limitations placed on the test by the customary technic must be disregarded in order to detect them.

In the Wassermann test when the total volume is 0.5 c.c., the difference between the average four-plus serum and the average control tube is at least 0.05 c.c. of complement, and it may be considerably more. The conditions surrounding the gonorrhea test are quite different. Many known positive serums cannot be made to show anything but a plus-minus result. In this case, the

*From the Wassermann Laboratory of the Department of Health.
Received for publication, April 10, 1935.

difference between the amount of complement fixed in a positive test tube and the amount absorbed by the control tube is very small.

In the classical gonorrhea complement fixation test the total amount of complement fixed is small even under the best conditions, therefore we are deprived of the latitude which exists in the Wassermann test whereby the positives can be graded between four-plus and negative. In gonococcus fixations, the total complement fixation for the average positive serums is seldom in excess of 0.02 c.c. of complement. This leaves little room for graduation of the positives except in occasional cases. The gonorrhea test therefore resolves itself into a problem necessitating the detection and quantitative estimation of exceedingly small amounts of complement.

Small differences in complement strength cannot be estimated accurately if the concentration of complement present is large. When large concentrations of complement are used in gonorrhea fixations, the large quantities left over make it impossible to detect the small amounts that have been fixed. For accurate quantitative determinations of complement, its concentration must approach the threshold value as indicated in our paper on "The Inadequacy of Present Complement Titrations."[2] Therefore, in a proper gonorrhea fixation test, it is imperative that the readings be made at the point where the concentration of complement approaches its threshold value.

However, when the concentration of complement is reduced, the test cannot be completed in the usual manner because the control tubes will be anticomplementary and the reaction tubes will not show definite positive or negative reactions. In addition, most positive gonorrhea serums do not completely fix the complement even though it is present in small amounts.[3] As a result, these positive specimens show a trace of hemolysis with the ordinary 5 per cent cell suspensions. This hemolysis disguises the true positives.

A properly planned titration demonstrates how these difficulties may be overcome. To a series of tubes, each containing an equal amount of complement, are added graded amounts of sensitized cells. The volumes of the reacting solutions are equalized with normal saline. The complement is of such strength that only the tube having least cells is completely hemolyzed. From this it is seen that a small amount of complement can completely hemolyze a cell suspension when the cell density is reduced. Furthermore, the supernatant fluids of the succeeding tubes show that although a given amount of complement may be insufficient to hemolyze completely a light cell suspension, it will produce more hemolysis when it reacts with cell suspensions of greater density.

Therefore when the first requisite of gonorrhea fixation tests is fulfilled, i.e., the use of reduced concentration of complement, to get clearer control tubes and clear cut negative reactions, the cell suspensions must be lighter than those used in present work. Furthermore, as the density of the suspensions is decreased, hemolysis disappears in the positive reaction tubes, and definite positive reactions are at last attained.

We have found that when one-fifth of the reacting complement fixation solution is made up of sensitized cells, highly satisfactory results are obtained. The sensitized cell suspension consists of one part of 5 per cent cells and one and one-half parts of hemolysin of the proper titer.

It has been demonstrated that unless the amounts of nonspecific complement absorption of any and every serum are taken into consideration, the results are not of much value in truly quantitative fixation determinations.[2] The standard that determines our readings is that complement fixation should be judged by the difference between the amount of complement absorbed nonspecifically in the control tube and the amounts fixed both nonspecifically and specifically in the reaction tubes.

When a series of duplicate complement fixation tests are run with the same positive specimen, each test differing from the other in the amounts of serum used, it will be seen that fixation is not directly proportional to the amounts of serum present. In other words, when one tube has twice the serum of the second, the first tube does not necessarily have twice the fixation of the second. Also, when the fixations of weakly positive serums are compared with one another, it is seen that doubling the serums in one case may double the fixation, while with another doubling the amount of serum may not increase the fixation at all.

An essential difference between the proposed test and the classical is that we do not attempt a quantitative estimation of complement fixation by varying the amount of serum, but by changing the concentration of the complement in the hemolytic system, and by estimating the amount of fixation by the threshold value of the complement left over. In this new test we take advantage of the fact that in hemolytic systems, complement acts according to its concentration rather than its absolute quantity.[2]

THE LABORATORY SET-UP FOR THE TEST

TABLE I

THE COMPLEMENT TITRATION
INCUBATION AT 37° C. FOR ONE-HALF HOUR

Pooled serum	0.05	0.05	0.05	0.05	0.05	0.05
Complement	0.05	0.06	0.07	0.08	0.09	0.10
Normal saline	0.05	0.04	0.03	0.02	0.01	0.00
Normal saline	0.82	0.82	0.82	0.82	0.82	0.82
Sen. cells	0.23	0.23	0.23	0.23	0.23	0.23

That quantity of complement to be used in the tests is the amount which causes almost complete hemolysis. The proper tube will have a trace of cloudiness caused by cells that have not been hemolyzed. The complement is then diluted so that 0.1 c.c. contains that amount indicated by the titration.

TABLE II

THE COMPLEMENT FIXATION TEST FOR GONORRHEA
INCUBATION AT 37° C. FOR ONE-HALF HOUR

	CONTROL	I	II	III
Serum	0.05	0.05	0.05	0.05
Complement	0.1	0.1	0.1	0.1
Antigen	0.0	0.1	0.1	0.1
Normal saline	0.82	0.6	0.4	0.2
Sen. cells	0.23	0.2	0.15	0.1
Total volume	1.15	1.0	0.75	0.5

It is essential that the amount of antigen used is an excess. Additional antigen should not increase the amount of fixation of the positive specimens.

For ease of manipulation, following the first incubation, the saline and sensitized cell suspension should be mixed together and added by a single pipetting and the specimens reincubated. This also makes for increased accuracy.

INTERPRETATION OF RESULTS

The tests are read after the cells have settled to the bottom of the tubes. A test can be read only when the control tube is almost completely hemolyzed. When it is completely hemolyzed, too much complement has been used, and the test has to be repeated. When the control has much more than a trace of cells left over, the test cannot be read for the positive cases. These cases will have to be repeated. When the amount of complement for the serums is well chosen, all the tests can be read except those which are markedly anticomplementary.

– Reaction. The first reaction tube is as much hemolyzed as the control.
+ Reaction. The first reaction tube colorimetrically shows definite fixation against the control. The second reaction tube approaches complete hemolysis.
2+ Reaction. The first reaction tube has only a faint trace of hemolysis or is completely fixed. The second reaction tube has much hemolysis.
3+ Reaction. The first reaction tube is completely fixed. The second reaction tube has a faint trace of hemolysis or is completely fixed. The third reaction tube has definite hemolysis.
4+ Reaction. All the reaction tubes are completely fixed.

The test becomes valueless when the antigen is anticomplementary. It is satisfactory if after a fixation with a negative serum, the first reaction tube is as much hemolyzed as the control. Serums tend to reduce the anticomplementary properties of the antigen, and for that reason, to ascertain whether an antigen is suitable from this standpoint, fixations must be run with negative serums.

The effects of cell fragility are minimized in this test because the factors that affect the control, affect the reaction tubes identically. Since the reaction tubes are read against the control, it makes no difference whether the cells are resistant or fragile to the action of complement.

The test is very delicate and errors in technic show up immediately. When the complement is of too high a concentration, the control tubes are completely hemolyzed; and when it is of too weak a concentration, the control tubes have too little hemolysis to permit reading the fixation of the positive specimens.

SUMMARY AND CONCLUSION

We believe that we have developed a sensitive, truly quantitative, and stable method for estimation of complement fixation.

The test is sensitive because with a reduction in the concentration of complement used, clear cut definite positive and negative reactions have been attained.

It is quantitative because the amount of nonspecific complement absorption for every specimen is accurately calibrated and only the specific fixation is read;

and because equal quantities of serum are used in the control and in each of the reaction tubes.

Finally more uniform results are obtained by this method since the effects of cell resistance to complement action are minimized; and also since the effects of variation in cell suspension density are minimized, due to the reading of the reactions at the threshold values of the complement.

The principles underlying this test are applicable to other fixations.

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THE INADEQUACY OF PRESENT COMPLEMENT TITRATIONS*

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THE unit of complement is defined as the smallest amount of complement which will completely hemolyze 0.1 c.c. of 5 per cent sheep cells in the presence of an excess of hemolysin after an hour's incubation at 37° C. Despite the clarity of the definition, this hemolytic unit of complement is an indefinite and variable quantity. Complement fixation determinations which depend upon it for their accuracy are similarly affected. The reason for this is that the several factors involved in the determination of such a unit are variable and beyond laboratory control.

For example, the density of the cell suspension decides the size of this unit, for when the suspension is heavy, the unit is larger than when the suspension is light. Yet it is difficult if not impossible to make up suspensions of constant density. Efforts[1, 2] to produce a standard 5 per cent cell suspension based on colorimetric nepholometry and cell counts have been abandoned when experiments have shown them faulty.

Even if standard suspensions were achieved, the problem would not be solved because it is not possible to pipette absolutely uniform amounts of cells into a series of tubes from any suspension. In addition to the technical difficulties of pipetting equal volumes of fluid, there is the factor of the cells constantly settling out in the stock solution and even in the pipette. This makes standard cell suspensions theoretical rather than actual. Therefore, all methods of estimating complement strength and all complement fixations which require standard suspensions are subject to variation. In order to achieve accuracy, complement titrations and fixations must be independent of the inevitable variations in cell suspension density.

*From the Wassermann Laboratory of the Department of Health.
Received for publication, April 10, 1935.

Another obstacle to establishment of a standard complement unit is the difficulty of determining the point of complete hemolysis. Brooks[1] recognized this and proposed the use of the point of 50 per cent hemolysis as a criterion of the amount of complement present. Wadsworth, Maltaner and Maltaner[2] state, "The titration of complement to the point of complete hemolysis for the unit value, as compared with titration to the point of 50 per cent hemolysis, proved so inaccurate as to obscure completely the true quantitative relations that exist in the course of the reaction." Although they found that reading from 50 per cent hemolysis is much more accurate than reading from the point of complete hemolysis, nevertheless this advancement is not sufficient to bring the test to the degree of accuracy required by this type of work.

While this method avoids the necessity of determining the point of complete hemolysis, it is not free from the objection cited above, namely, the impossibility of preparing and pipetting a standard cell suspension.

Any such method contains other and equally serious faults. In hemolytic titrations when there is sufficient complement to produce approximately 50 per cent hemolysis, the addition of relatively large variations in the amount of complement produce small changes colorimetrically. Moreover, the eye is unreliable when called upon to distinguish or estimate these differences in the amounts of hemolysis, even when they are considerable. The amounts of complement which produce these undetectable changes in the degrees of hemolysis are nevertheless sufficient to produce wide variation in the results of complement fixation tests. In other words, this type of titration is not sufficiently sensitive and is of limited value in keeping complement fixation results accurate or stable.

The following work leads to a better estimation of complement strength:

When supernatant fluids of a complement titration are examined in a colorimeter, it is found that the amount of hemolysis obtained is not directly proportional to the amount of complement present. When complement is of such a concentration that 0.1 c.c. produces a slight amount of hemolysis, 0.2 c.c. produces more than twice that amount. The stronger the complement, the smaller are the differences in hemolysis caused by 0.2 c.c. and 0.1 c.c. complement, and conversely, the weaker the complement, the greater are these differences. Therefore, by looking at one tube of a titration it is impossible to predict what will happen in any other tube.

In an experiment of this kind when the hemolytic solutions were examined in a colorimeter, the solution containing 0.2 c.c. of complement had four times as much hemolysis as the tube containing 0.1 c.c. of complement. Doubling the amount of complement increased the color of the hemolytic solution four times. Since the addition of the second tenth of a centimeter of complement produced three times the amount of hemolysis as the first tenth, apparently only one-third or 0.033 c.c. of the first 0.1 c.c. of complement produced any color, and 0.067 c.c. of it was used up in overcoming the resistance of the cells. This was shown true when a tube containing 0.067 c.c. of complement failed to produce hemolysis. Applying the same reasoning to any hemolytic system, one concludes that only part of the complement present produces the hemolysis. The amount of complement used up in overcoming the resistance of the cells to

hemolysis plays an important part in estimating complement strength. In this paper, this amount of complement will be referred to as the threshold value of the complement and is represented in the titration by the largest amount of complement which may be added to the hemolytic system without producing any hemolysis.

Choosing any two tubes of a titration and applying to them the equation listed below, the result will always be the threshold value of the complement.

$$A - \frac{B - A}{X / Y} = \text{the threshold complement value}$$

A smaller amount of complement
B larger amount of complement
X colorimetric reading for the solution containing the smaller amount of complement
Y colorimetric reading for the solution containing the larger amount of complement

Like standard cell suspensions, this formula is theoretical rather than actual. It is applicable for tubes that have no more than 50 per cent or less than 10 per cent hemolysis. The factor which causes this limitation and also adversely affects the formula is that as cells hemolyze because of the action of the complement, the resultant hemolysis inhibits further action of the complement. This will be discussed shortly. The formula is also affected by the fact that no two tubes contain the same amounts of cells, and by the technical difficulties of pipetting exact amounts of complement, and by our ability to read colorimeters, and by the difficulty of judging where hemolysis begins. Because of all this, the figure obtained by the equation, instead of checking perfectly, may approximate the largest amount of that complement that may be added to the hemolytic system without producing any hemolysis.

The fact that any two tubes of a titration when resolved according to the above equation always point to the threshold tube—i.e., the tube containing the most complement which has no hemolysis—makes this tube the most stable in any titration. In actual practice it is easier to find the threshold value than to predict it.

It is for several reasons that the tube which contains the least amount of complement above the threshold value, i.e., the first tube that shows hemolysis, is the most important in any titration. In this tube small changes in the amounts of complement produce easily detectable variations in the amounts of hemolysis. Amounts of complement that produce no visible changes when hemolysis is 50 per cent or more, produce startling changes where hemolysis begins. Another justification for the reading of complement titrations from this tube is that small changes in the density of the cell suspension which would markedly affect the point of 50 per cent hemolysis, have almost no effect on the tube which shows little hemolysis. Therefore, by using the least hemolyzed tube of a titration to estimate complement strength there are two positive advantages to be gained; one is that small changes in complement strength are easily detected and the other is that the effects of cell density variation are minimized.

Aside from these advantages the following experiment reveals additional considerations for this type of titration reading. Taking account of the fact that complement acts according to concentration and not to absolute quantity,

a titration may be made with constant amounts of complement and sensitized cells but with increasing amounts of salt solution. This kind of titration shows that it is possible to make any quantity of complement produce any amount of hemolysis by simply changing the volume of solution in which it is allowed to act. And so it is possible to obtain more hemolysis with 0.1 c.c. of complement in a small reacting volume than with 0.2 c.c. in a volume so large that its concentration is less than that of the 0.1 c.c. This important fact has hitherto not been used to its fullest advantage in complement fixations.

We slightly digress to note that in systems consisting of patient's serum, bacteriolytic antigen, and complement, the complement acts according to the absolute quantity present rather than according to its concentration. Of a given amount of complement, the same quantity is fixed specifically by the immune substances whether the reacting volume is large or small. We have found this to hold true in Wassermann and gonorrhea fixations. This definitely establishes the fact that bacteriolytic systems are different from hemolytic.

We can demonstrate that as hemolysis proceeds in any tube, the internal osmotic pressure of the cells falls because of the loss of electrolytes from the cells. As the process goes on, the electrolytes lost by the cells are added to the outside solution which is thereby raised in osmotic pressure until the inside and outside pressures of the cells are in equilibrium. The more hemolysis there is in any tube, the greater is the resistance effort of the suspension against further action of the complement. Any tube that shows considerable hemolysis does not represent the absolute potency of the complement present.

From this it is seen that it is a mistake to assume that because graded amounts of hemolysis are obtained with graded amounts of complement hemolysis ceases when the complement is used up by the hemolytic system. In every tube of a titration when hemolysis stops, there is still some free complement, but its concentration is too low to produce any more hemolysis.

Returning to the estimation of the value of a faintly hemolyzed tube, it will be seen that in addition to the advantages listed above, it also has the advantage of not being affected by the back pressure produced by a solution containing hemoglobin and expelled electrolytes. In fact, it is the only tube in a titration which can be depended upon to give a true indication as to the strength of the complement.

A final consideration is that complement titrations, no matter how made or evaluated, are influenced by cell resistance to complement action. In any titration, it is difficult to determine to what degree the amount of hemolysis obtained is influenced by this fact. The same picture is obtained when the complement is strong and the cells resistant as when the complement is weak and the cells fragile. This is perhaps the most disturbing factor encountered in complement fixation work and the results of all complement fixation reactions are influenced when cells of the two extremes are used. When complement is titrated for its hemolytic activity without regard to its bacterio-fixing value, as is commonly done, and when different lots of cells are used on successive fixations with a positive specimen, different values will be obtained for specific complement fixation. When the cells are weakly resistant to hemolysis, more

immune substances will be credited to the specimen than when the cells are strongly resistant to hemolysis.

The effects of variation of cell resistance to hemolysis can be minimized by a titration of complement against a standard border-line four-plus serum. A second titration should be run to determine the density of the cell suspension that would give complete hemolysis with negative specimens.

This sort of titration would tend to keep results uniform, but like all other present titrations, fails to take into account anticomplementary substances any specimen may contain. When, regardless of the age of the serums, sufficient anticomplementary substances are present so that 0.08 c.c. of any serum is capable of absorbing from 5 per cent to 100 per cent or more of that complement necessary to hemolyze completely 0.1 c.c. of a 5 per cent cell suspension, titrations are not of much value in truly quantitative fixation determinations unless they indicate the amounts of complement nonspecifically absorbed by each and every specimen.

Complement fixation by serum and antigen above the amounts absorbed by the anticomplementary substances is specific complement fixation when the antigens are not anticomplementary. When the sole difference between the reaction tube and the control tube is the lack of antigen, and when hemolysis approaches completion in the control tube, any lack of hemolysis in the reaction tubes is due to specific fixation. The quantitative determination of this is important and will be discussed in a later paper.[2]

SUMMARY AND CONCLUSIONS

Titrations and fixations must be read where hemolysis begins, so that they will be independent of the inevitable variations in cell suspension density; and so that they will be sensitive—for amounts of complement that produce no changes where hemolysis is appreciable, produce detectable changes where hemolysis begins; and finally, so that the resistance to further complement action, engendered by hemolysis, will be minimized.

Furthermore, systems that do not take into account the amounts of nonspecific complement absorption of every specimen, fail to be quantitative.

We have taken advantage of the material briefly outlined in this paper, and as a result, think we have succeeded in developing more sensitive fixation tests.[2]

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A METABOLISM CHAMBER WHICH AUTOMATICALLY MAINTAINS A CONSTANT PARTIAL PRESSURE OF OXYGEN*

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IN 1850 Regnault and Reiset[1] described an apparatus for measuring the respiratory exchange of animals, consisting of a closed chamber in which the animal was placed, a system for circulating the air and removing the carbon dioxide and water vapor excreted, and a means for automatically supplying oxygen to the chamber to replace that absorbed by the animal. The principle of this so-called "closed circuit apparatus" has been employed in numerous instruments devised for the study of respiration, metabolism, and anoxemia, notably the calorimeters of Atwater and Benedict and of Lusk, the clinical metabolimeters of Krogh and of Benedict, and the rebreather used in the examination of government aviation personnel.

In the usual laboratory form of this apparatus the air of the animal chamber is circulated continuously by a pump, passing through soda lime which absorbs the carbon dioxide, and either sulphuric acid or an ice chamber, which removes the water vapor. The subtraction of these gases decreases the pressure in the chamber; this causes an equivalent volume of oxygen to flow in from a gasometer which is at atmospheric pressure, thus restoring the chamber pressure to normal. However, this closed system of chamber and absorbers is in effect a combined gas-thermometer and gas-barometer, a rise in temperature or a drop in barometric pressure causing increased pressure within the system, and vice versa. Since the only communication between the system and the atmosphere is through the gasometer, such pressure changes must interfere greatly with the normal flow of oxygen into the system, causing correspondingly large variations in partial pressure of the oxygen in the animal chamber. For example, the combined effect of the extreme but possible atmospheric changes of +20° C. and −40 mm. Bar. is to increase the pressure or volume of a gas 10 per cent. This is of little importance in experiments where the partial pressure of oxygen in the animal chamber is 300 to 500 mm. Hg, but in studies requiring an oxygen tension of 75 mm. Hg or less, changes of such magnitude might double the tension or reduce it to zero. Hence, frequent gas analysis and constant adjustment of the oxygen supply are necessary in order to maintain the oxygen tension at the desired level.

The present apparatus was designed to compensate for temperature and pressure effects, and to eliminate the necessity for constant supervision. It is shown diagrammatically in Fig. 1. Chamber *A* is occupied by the animal. Air circulates from the top of this chamber, through the carbon dioxide absorbers *D*, the motor-driven pump *F*, the ice chamber (water remover) *G*, and back

*From the Wilmer Institute of the Johns Hopkins University and Hospital.
Received for publication, April 11, 1935.

to the bottom of the chamber. On either side of the animal chamber are the compensating chambers *B* and *B'*, which are closed to the atmosphere and the animal chamber but are open to the tank *C*. By running water into this tank

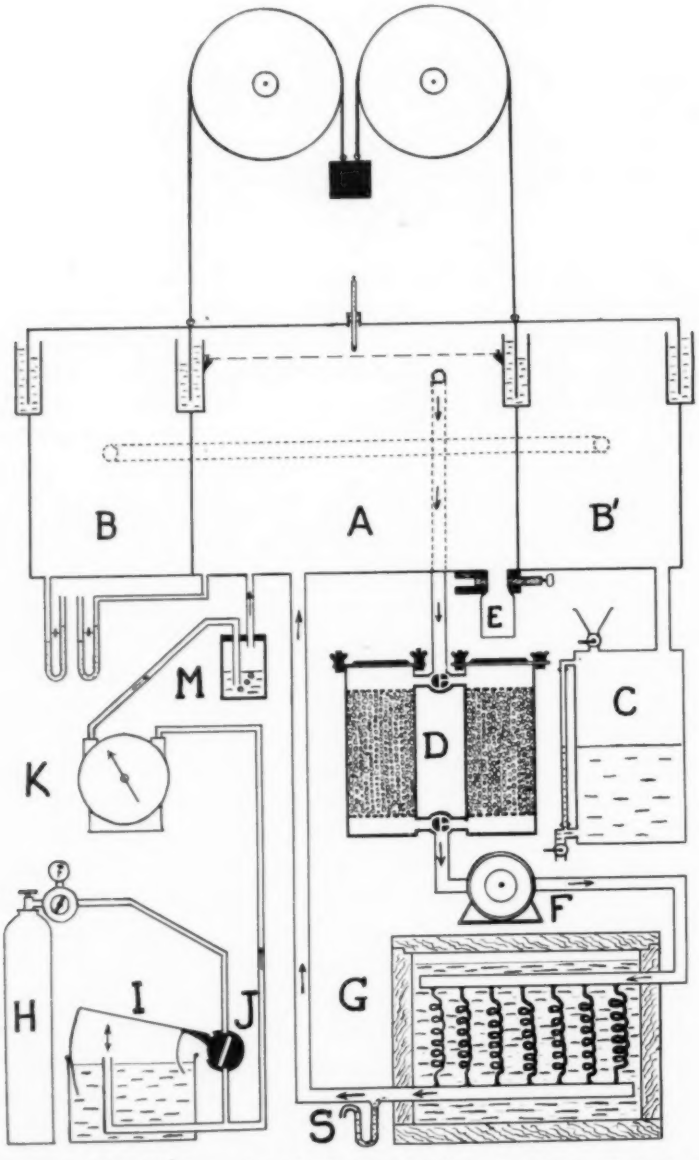

Fig. 1.—*A*, animal chamber. *B* and *B'*, compensating chambers. *C*, tank for adjusting air volume of compensating system. *D*, carbon dioxide absorbers. *E*, glass jar attached to sliding valve, for collecting excreta. *F*, air circulating pump. *G*, ice chamber for condensing water vapor. *S*, syphon trap. *H*, oxygen cylinder with reducing valve. *I*, oxygen gasometer. *J*, automatic oxygen valve. *K*, oxygen meter. *M*, Mueller valve.

the gas volume of the compensating system may be adjusted to equal that of the animal chamber and absorbing system. The cover, common to the animal chamber and the compensating chambers, is carefully counterbalanced and

moves freely up and down like the bell of a gasometer. The area of the cover of *A* equals the combined areas of the covers of *B* and *B'*. Both systems have valves opening to the atmosphere for use in adjusting the chamber pressures, and each system is provided with a water manometer indicating the chamber pressure relative to that of the atmosphere.

At the start of an experiment the animal is placed in chamber *A* and the cover lowered until its side walls are half submerged in the water-seals. The valves to the atmosphere are then closed, the pump started, and by manipulating one of the valves air is withdrawn from the animal chamber until its manometer reads −10 mm. Since the two chamber systems contain equal volumes of gas and have covers of equal area, the mechanical effect of the minus pressure, transmitted through the cover to the gas in the compensating system, causes the manometer of chamber *B* to read +10 mm. The Mueller valve *M* has a resistance of about 10 mm. of water, and as the animal's absorption of oxygen increases the negative pressure in *A* this resistance is overcome and oxygen flows into the system from the gasometer *I*, through the oxygen meter *K*. In the usual apparatus any change in room temperature or barometric pressure interferes with the normal inflow of oxygen. In the present apparatus, however, the gas in the compensating system expands or contracts equally with that in the animal chamber system, the cover rises or falls to compensate for the change in volumes, and the pressure relations remain unaltered. Hence the negative pressure in the animal chamber varies little, oxygen flows in according to the needs of the animal, and the oxygen tension is maintained within narrow limits.

Theoretically the variation in oxygen tension in the chamber should be no greater than that of the atmosphere. Practically it is somewhat larger, owing to the friction of moving parts of the apparatus and the difficulty of eliminating small leaks. Nevertheless, a series of three-day runs during periods of very changeable weather indicates that the oxygen tension in the chamber may be kept within limits of ±3 per cent.

Details of Construction and Operation.—The chamber and cover are made of 18 gauge galvanized iron, carefully soldered at the seams. The animal chamber is 90 cm. long, 75 cm. wide, and 70 cm. deep, and has a volume of about 500 liters. The compensating chambers have similar vertical and lateral measurements but are only one-half as long, making their combined volume equal to that of the animal chamber. The water-seals are 20 cm. deep, and the side walls of the cover are 22 cm. high. The effective volume of each cell of the cover is approximately one-fifth of that of the corresponding chamber. The cover and counterweight are suspended on 18 gauge piano wire from a pair of bicycle wheels mounted on the ceiling. Change of cover weight due to its immersion in the water-seals was originally compensated for by the use of a mercury syphon attached to the counterweight, but this refinement was found unnecessary in practice. A vertical scale on the cover, calibrated in terms of volume, is used when making gas mixtures in the chamber.

Oil-impregnated maple set in petroleum pitch covers half of the animal chamber floor, and provides a comfortable resting place for the animal. There

is a large plate-glass window in the front of the chamber, and a smaller one in the cover. At the top of the animal chamber is a grill (Fig. 1) which prevents the animal from striking its head against the cover. All connections between the chambers, tank, and absorbers are made with standard one-inch iron pipe, well painted at the joints to prevent leaks.

Since the animal usually remains in the chamber for several days, provision has been made for removing excreta and supplying food and water without raising the cover or otherwise changing the oxygen tension appreciably. In one corner of the chamber floor, there is an opening 7.5 cm. in diameter, fitted with a sliding gate valve. The gate is made of 3 mm. German silver, the rubbing surfaces are ground to a careful fit, and uniform pressure between parts is maintained by means of springs. This type of valve is not gas-tight, but in the present situation the valve is always wet and moisture drawn into the crevices by capillary action provides a perfect seal for the pressures in use. The bottom of the valve is threaded to receive a screw top glass jar (*E*, Fig. 1), which seats tightly against a soft rubber gasket. Immediately above the valve opening, the side walls of the animal chamber have been cut away

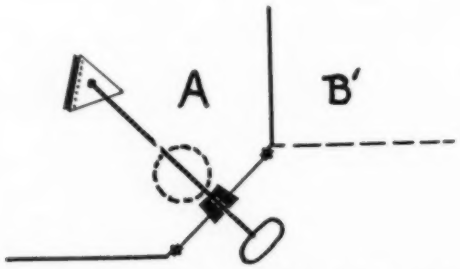

Fig. 2.—Plan of rubber window and sliding rake, for cleaning animal chamber.

(Fig. 2) to permit the placing of a round window 15 cm. in diameter, set perpendicular to the floor and at an angle of 45° to the long dimension of the chamber. The adjacent corner of the compensating chamber is also cut away to accommodate this construction. The window is made of soft sheet rubber 5 mm. thick, and in its center a large rubber stopper is fixed. The stopper is pierced with a 6 mm. hole through which passes the metal handle of a rake. The handle is lubricated with petrolatum and slides easily through the stopper without causing leaks. The blade of the rake is a triangle of steel, and along one edge is fixed a strip of rubber. The sharp edges of the rake dislodge adherent matter, and the soft rubber edge is used to shift semiliquid material. Since the window is set at an angle of 45°, it is possible to reach any part of the chamber floor, and to rake solid excreta and débris to the valve opening where it falls into the glass jar. When the jar is full, the valve is closed and the jar is removed and replaced by another. Ordinarily the valve remains open, and since the floor slopes from all directions toward the valve opening, urine drains into the jar as soon as it is deposited.

Food is introduced into the chamber by the mechanism shown in Fig. 3. The food carrier is a metal cylinder 18 cm. long and 7.5 cm. in diameter, closed at the ends, fitted with a leather compression ring near each extremity, and

attached to a handle. It is open at one side to receive the food. Lubricated with petrolatum, it slides in the tightly fitting outer tube which passes through the side wall of the chamber. When the carrier is at the outer end of its stroke, ready to receive food, the seal is maintained by the inner compression ring; when it is at the inner end of the stroke the greater part of the carrier is within the chamber and the seal is maintained by the outer compression ring. In this position, the carrier is rotated 180° by means of the handle, and the food drops on the chamber floor. Water is automatically supplied to a drinking trough through a syphon arrangement.

The carbon dioxide absorbers (*D*, Fig. 1) are metal cylinders 22 cm. in diameter and 45 cm. high, fitted with covers sealed by rubber gaskets. Each holds about 20 pounds of 4-8 mesh soda lime. A screen at the top serves to trap any hair or other matter which may be drawn in from the animal chamber. On the bottom screen is a thin layer of cotton which prevents lime dust from leaving the absorber. A pair of three-way valves, worked simultaneously by the same lever, permits one absorber to be refilled while the other is in operation.

The pump (*F*, Fig. 1) is an eccentric-type positive blower, directly con-

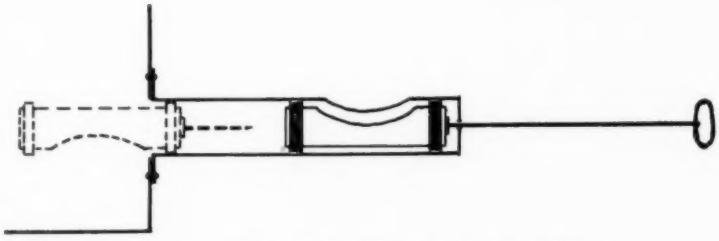

Fig. 3.—Elevation of food carrier mechanism.

nected to a variable speed ½ H.P. electric motor running at a speed of 450 r.p.m. The ventilation rate of 500 liters per minute keeps the carbon dioxide and humidity within normal limits.

The ice chamber or water remover (*G*, Fig. 1) is a metal box 60 cm. long, 45 cm. wide, and 75 cm. deep, insulated with a 7.5 cm. layer of sheet cork on all sides, and containing 6 vertical coils each consisting of 1.5 meters of 1 cm. copper tubing. These coils are connected to the inflow and outflow pipes at top and bottom. The chamber is filled with ice and ice water, and as the circulating air passes through the coils, water is condensed out of it and escapes to the drain through the syphon *S*. The outflow pipe is insulated with cork, and the air returns to the animal chamber cool and comparatively dry.

The Mueller valve (*M*, Fig. 1) is of the usual type. The depth to which the tube is immersed in the water determines the negative pressure in the animal chamber; therefore the tube should have an internal diameter of at least 1 cm., and V-shaped notches filed in its walls at the tip so that the oxygen may escape into the water in small bubbles.

The oxygen meter (*K*, Fig. 1) is the usual rotary wet meter; it should be kept filled to the mark with water, and calibrated periodically against a standard gas holder.

The gasometer (*I*, Fig. 1) is of the segment type; the bell is 10 cm. wide and 45 cm. long, is counterbalanced, and moves freely on ball bearings. Its shaft operates valve *J*, thus controlling the flow of gas from the reducing valve of the oxygen cylinder *H*. As oxygen is drawn from the gasometer by the negative pressure in the animal chamber the bell descends, opening valve *J* and allowing oxygen from the cylinder to flow into the system. Any excess over the amount required in the animal chamber passes into the gasometer bell, causing it to rise, thereby closing valve *J* and cutting off the flow of oxygen from the cylinder. The gasometer is, in effect, a second reducing valve, simple and very sensitive, and this arrangement supplies oxygen automatically until the cylinder is empty.

This apparatus was designed for the rather special purpose of studying animals under conditions of lowered oxygen tension, and the metabolism was calculated on the basis of the oxygen consumption, without determining the respiratory quotient. More precise results would doubtless be obtained if the wet meter were kept in a constant-temperature box; and the respiratory quotient could be determined by substituting the usual sulphuric acid-soda-lime-sulphuric acid train for the present carbon dioxide absorbers and ice chamber. If this system were adopted, the water-seals of the cover should be filled with mineral oil.

SUMMARY

A new apparatus for the study of gaseous metabolism is presented, incorporating the closed circuit principle of Regnault and Reiset, with means of automatically compensating for the effects of temperature and barometric pressure on the oxygen tension in the animal chamber. Mechanisms for supplying food and removing excreta without appreciably altering the oxygen tension, and of automatically delivering oxygen from a high pressure cylinder to the animal chamber, are described and illustrated. The oxygen tension is maintained within ±3 per cent, as checked by frequent gas analysis, and an animal may live comfortably in the chamber for several weeks.

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DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

HEMOPHILIA, Histopathology of Hematopoietic Tissues in, Custer, R. P., and Krumbhaar, E. B. Am. J. M. Sc. **189:** 620, 1935.

Three fatal cases of hemophilia with necropsy are reported, 1 patient dying from uncomplicated hemorrhage, 1 from appendicitis and hemorrhage, and 1 from fulminating pneumonia.

The hemopoietic tissues all showed normal regenerative ability, the first two predominantly erythroblastic, the third leucoblastic.

All three showed a marked increase of megakaryoblasts and megakaryocytes in the bone marrows, indicating a relationship of the blood platelets to the hemophilic process.

J. H. Wright's observation of platelet formation in the bone marrow sinusoids from intruding pseudopods of megakaryocyte cytoplasm was supported by the findings.

EOSINOPHILIA, in Scarlet Fever, Friedman, S. Am. J. Dis. Child. **49:** 933, 1935.

An eosinophile count was made in 100 cases of scarlet fever in the early stages of the disease. In 46 per cent of the cases eosinophilia was demonstrated in a single count.

Eosinophilia is most likely to be present in the early stages of the disease in the mild cases in which there are mild constitutional symptoms and a mild rash. The incidence of eosinophilia in such cases was from 60 to 70 per cent as compared with 46 per cent in all cases of the disease. It is in the mild type of case that frequently there is difficulty in making the diagnosis. The use of the presence of an elevated eosinophile count as an aid to diagnosis is recommended.

PNEUMONIA, Incidence of Bacteriemia in and Its Relation to Mortality, Bullowa, J. G. M., and Wilcox, C. Arch. Int. Med. **55:** 558, 1935.

The incidence of bacteremia in connection with the fatality rate is listed for different types of pneumococcic pneumonia in a series of 1,725 cases due to a single type, occurring over a five-year period, at Harlem Hospital.

The percentage of invasion of the blood differs for the different types, though in the main the percentages of invasiveness and mortality are approximately the same.

The importance of (1) differentiating the types formerly included as group IV or the x group and (2) of typing individual cases for prognosis and eventual treatment, when specific treatment is available, is manifest.

Only 3 per cent of pneumococcic pneumonias escape classification at the present, when typing is carried from I to XXXII (Cooper).

The variation in percentage of invasion and fatality from year to year in different pneumococcic types is evidence of the variations which are encountered from season to season, and shows the necessity of extending over several years the testing of any proposed curative substance.

INFECTIONS, by Gas-Forming Anerobic Bacilli, Reeves, J. R. J. A. M. A. **104:** 526, 1935.

Approximately 30 per cent of cases reported and treated as *Clostridium welchii* gas gangrene are caused by other anaerobic organisms.

This 30 per cent of cases should be classed as putrefactive gangrene and treated conservatively by systemic supportive treatment, débridement and irrigations. *Clostridium welchii* antitoxin is not indicated and may be injurious.

Putrefactive gangrene is likely to appear in patients past the age of fifty years who are constitutionally below normal. Cases appear notably in patients with circulatory failure, arteriosclerosis, thromboangiitis obliterans and diabetes mellitus.

The direct cause for putrefactive gaseous lesions in the muscles of patients whose condition has been described may be bruises, burns, simple fractures, parenteral administration of drugs and solutions, and circulatory failure.

Putrefactive gas-forming anaerobic soil bacteria contaminate food at all times. It seems apparent that bacteria of this type are frequently present in the organs and muscles of the aged individual.

Diagnosis of *Clostridium welchii* infection cannot be based on direct smear; the rabbit inoculation test or identification is best corroborated by the use of anaerobic cultural methods and related criteria.

Patients suffering from wounds, particularly of muscle tissue, which have been contaminated with soil or street dirt, should be given gas gangrene antitoxin prophylactically and in addition should receive débridement and irrigations. If toxic symptoms develop radical débridement, continuous irrigations, and therapeutic serum are indicated without delay.

TULAREMIA, Kavanaugh, C. N. Arch. Int. Med. **55**: 61, 1935.

In addition to a short review of the literature, the characteristic clinical features in 123 cases of tularemia are presented, together with observations at autopsy in one case.

A new classification is presented. Sixty-nine cases occurred in males and 54 in females. One hundred and eight were of the primary cutaneous type, 6 were of the primary ophthalmic type and 9 were of the cryptogenetic type of the disease.

The average incubation period, definitely determined in 58 cases, was four and one-half days.

Sixteen cases presented pulmonary involvement. Primary and secondary pulmonic tularemia are discussed. The breast was involved in 2 cases. Thrombi in the veins were noted in 1 case. Pleurisy was a complication in 6 cases. Abdominal symptoms were prominent in 5 cases. Peritonitis was present in 1 instance. Delirium was a prominent feature in 6 cases. Acute mania complicated 1 case. In 1 case of the primary ophthalmic type optic atrophy occurred, with loss of vision in the affected eye. A purulent dacryocystitis complicated 1 case. Decided splenomegaly with perisplenitis was present in 5 instances. Osteomyelitis was observed once. Cervical adenitis as the only external evidence of the disease was observed in 4 cases. Subcutaneous nodules occurred in 28 instances. A cutaneous eruption was present in 23 cases (18.7 per cent).

There were 5 deaths, indicating a mortality of 4 per cent in this series.

Other pertinent facts pertaining to the history, epidemiology, pathology, diagnosis, and treatment are also discussed.

RETICULOCYTES, Fragility and Maturation of, Mermod, C., and Dock, W. Arch. Int. Med. **55**: 52, 1935.

The reticulocytes, and particularly the young (heavily stained) ones, are more easily destroyed in shed blood and in the circulating whole blood by saponin (and by citrate and oxalate in whole shed blood) than are the other red cells.

The maturation of reticulocytes in the circulating blood is not proved; it seems not to occur on any significant scale.

The occurrence of a reticulocytic crisis early in the treatment of anemia is probably an unavoidable evil associated with a change in the structure and function of the bone marrow. This precedes and delays the rise in the red cell count.

ENCEPHALOPATHY, Lead, Simulation of Intracranial Tumor, Bucy, P. C., and Buchanan, D. N. J. A. M. A. 105: 244, 1935.

In two of three cases of lead encephalopathy in children with increased intracranial tension simulating cases of intracranial tumor, a suboccipital decompression was made. Both children recovered promptly and completely. In the third, in which the intracranial pressure was less elevated, no decompression was made and medical management alone was used. This child had a prolonged and stormy course but also ultimately recovered. From these experiences and from data taken from the literature, it is concluded that:

1. The differentiation of lead encephalopathy and midline cerebellar tumors in children from the history and physical signs alone is difficult and may be impossible.

2. Such factors as the possible ingestion of lead, rapid onset with vomiting and abdominal pain, and only moderately increased intracranial pressure should warn the clinician of the possible diagnosis of lead encephalopathy.

3. The easiest and most rapid method of rendering probable the diagnosis of lead encephalopathy is the demonstration of the presence of lines of increased density at the ends of the long bones. The diagnosis may then be established by demonstrating the presence of lead in the blood.

4. Medical management of lead encephalopathy is notoriously unsatisfactory and results in a high mortality and numerous and serious sequelae.

5. Surgical decompression of the intracranial cavity offers a logical means of producing a prompt and complete relief of symptoms with less danger of sequelae.

TYPHOID VACCINE, Study of Agglutinin Response to, Valentine, E., Park, W. H., Folk, G., and McGuire, G. Am. J. Hyg. 22: 44, 1935.

The rise in the agglutinin titers of the blood serums of rabbits and human beings, following the percutaneous administration of typhoid vaccine, paralleled the number of treatments and the size of the dose. The human beings gave small rises in agglutinin titer after a large number of rubbings; the rabbits with a greater number of rubbings than the human beings, larger doses, and a relatively greater rubbing area, gave good agglutinin titers.

Intracutaneous injections of typhoid vaccine in weekly doses of 50, 100, and 150 million bacilli produced as great an agglutinin response as subcutaneous injections of 100, 200, and 300 million, or 250, 500, and 1,000 million bacilli. Intracutaneous injections of 25, 50, and 100 million produced slightly less response.

No appreciable systemic reactions and only slight to moderate local reactions followed the intracutaneous injections. Subcutaneous injections in doses of 100, 200, and 300 million bacilli, even 250, 500, and 1,000 million, produced some local and constitutional reactions, but much less than is commonly found with the standard subcutaneous doses of 500, 1,000, and 1,000 million.

Oral administration of three different preparations of mixed typhoid-paratyphoid vaccines without preliminary bile treatment produced no significant rise in agglutinin titer when tested one month after ingestion.

These results suggest the greater use of the intracutaneous method to obtain immunization.

TUBERCLE BACILLI, Analysis of Loewenstein's Investigations, Siegel, M. Am. J. M. Sc. 190: 435, 1935.

Of 911 blood specimens from 422 tuberculous persons at this hospital, there were 6 (1.4 per cent) macroscopic positive cultures and 55 (13 per cent) microscopic positive cultures by the Loewenstein technic. The macroscopic positive cultures were undoubted cultures of tubercle bacilli according to subculture and virulence tests. The microscopic positive cultures were not definitely proved to be cultures of tubercle bacilli, since subcultures and animal inoculation gave negative results.

The disparity between Loewenstein's results and those of other investigators still exists after five years of research.

Loewenstein's investigations may be criticized from two points of view: his definition of a positive blood culture and his conception of a control.

BACTERIAL FLORA, Associated With Foreign Bodies in Trachea and Bronchi, Bucher, C. J. Am. J. M. Sc. **190:** 409, 1935.

Cultures seeded from pus bronchoscopically collected from the trachea and bronchi furnish more reliable data on the kind of microorganisms there, than those seeded from expectorated material.

The bacterial flora present in the air passages in which a foreign body is lodged does not materially change with the type of foreign body, its location or length of sojourn there, the age of the patient and degree of obstruction induced by it.

Other things being equal, one pathogen seems as capable as another of producing severe grades of infection in an obstructed tracheobronchial tree.

Quantitative differences in the numbers of colonies of different organisms on a plate of blood agar seeded from pus recovered from these lesions are a reliable guide in estimating the part that a given organism plays in the resulting infection.

The indications from a limited number of studies of the anaerobic bacteria from such lesions are that they are of minor importance.

The bacterial invasion and infection of the tissues of the lower respiratory tract at the site of a foreign body seem to follow the same general principles as those encountered in obstructive lesions elsewhere in the body. The irritation produced by the obstructing body itself and the amount of hindrance to drainage, primarily, and, secondarily, other factors favoring or hindering microbic multiplication such as the age and hardihood of the tissues, pressure on them and the duration of the irritation, determine and limit the infection.

SCARLET FEVER, Convalescent Serum in, Joyne, A. L., Levinson, S. O., and Thalhimer, W. J. A. M. A. **105:** 783, 1935.

Of 862 home contacts who gave no history of scarlet fever and were passively immunized with convalescent scarlet fever serum, scarlet fever did not develop in 97.2 per cent.

Of 83 Dick-positive hospital contacts immunized with convalescent scarlet fever serum, scarlet fever did not develop in 95 per cent.

In immunized contacts in whom scarlet fever developed, it was usually in a modified form, believed to have been produced by partial immunization and resultant sero-attenuation.

Convalescent scarlet fever serum in adequate therapeutic doses administered early may abort the disease and usually causes recession of fever, diminution of toxemia and angina, and fading of the rash and appreciably shortens the period of illness.

Convalescent scarlet fever serum, directly or indirectly, either prevented the development of complications or reduced the frequency of their occurrence.

The influence of serum on late and complicated cases was less marked but frequently seemed beneficial.

By reducing the severity of the disease and the incidence of complications, the mortality rate was definitely diminished.

No unfavorable reactions, serum sickness, sensitization or anaphylactic shock were encountered with the use of human convalescent scarlet fever serum.

PLAGUE, Rodent, in California, Kellogg, W. H. J. A. M. A. **105:** 857, 1935.

At present there is a lighting up of enzootic plague, which had become a commonplace, into a rather alarming epizootic, which is the most extensive outbreak of squirrel plague since the peak of the epizootic in Contra Costa and Alameda Counties in the period between 1907 and 1919. Other sharp outbreaks have doubtless occurred that burned themselves out with-

out the condition is being brought to attention in time to prove the nature of the epizootic. The present outbreak, however, has certain aspects that are not entirely reassuring. It is not without significance that after a considerable period of quiescence plague is found to be actively spreading among the wild rodent population of rural areas in widely separated districts and in areas far from any formerly known focus of infection. The prevalence of infected squirrels near the borders or Oregon and of Nevada and on the other side of the mountain range suggests that there is no natural limitation to the spread of plague through wild rodents to places far distant from its original entry into this country in the Bay district of California. Plague is very evidently a permanent problem on the Pacific Coast and the prospect of its becoming a problem in other states appears at the present time to be good. Especially to be feared so far as man is concerned is the pneumonic form of the disease, which, as has been indicated, may be directly related to plague in animals of the squirrel and ground hog type.

MYELOMA, Multiple, With Hyperproteinemia, Sweigert, C. F. Am. J. M. Sc. **190:** 245, 1935.

A case diagnosed clinically as multiple myeloma with hyperproteinemia is reported and the cases in the literature reviewed.

Hyperproteinemia is decidedly uncommon and occurs, in its most striking form, in multiple myeloma.

In the majority of instances it has been attributable to hyperglobulinemia without demonstrable relationship to Bence-Jones protein. In three cases it was due to extraordinary amounts of Bence-Jones protein in the serum. In the present case, both globulin and fibrinogen were increased in the blood plasma.

Hyperproteinemia may produce unusual and variable clinical phenomena, notably: difficulty in counting erythrocytes; autohemagglutination; markedly accelerated sedimentation velocity of the erythrocytes; abnormal coagulability of the blood and spontaneous precipitation of protein in drawn blood. In the presence of Bence-Jones proteinemia, precipitation in the serum may occur during inactivation for the Wassermann reaction.

The finding of hyperproteinemia or any of its manifestations should suggest multiple myeloma as a diagnostic possibility.

The clinical aspects of the blood and kidney changes in the present case are emphasized and their diagnostic importance is indicated. These include (a) a severe, progressive, macrocytic anemia with evidences of profound bone marrow disturbance and active regeneration, unassociated with achlorhydria and refractory to liver and iron therapy; and (b) an atypical nephropathy in which impairment of renal function without hypertension is a distinctive feature.

CEREBROSPINAL FLUID, During and Between Attacks of Migraine Headaches, Von Storch, T. J. C., and Merritt, H. H. Am. J. M. Sc. **190:** 226, 1935.

In 15 cases of migraine, the cerebrospinal fluid pressure, measured during a migraine headache, was slightly elevated in one case (190 mm. of water), slightly low in 3 cases (40, 50, and 80 mm. of water) and averaged 123 mm. of water.

In 29 cases of migraine observed during an asymptomatic interval, the cerebrospinal fluid pressure was slightly elevated in 2 cases (185 and 190 mm. of water), low normal in 3 cases (90, 90 and 95 mm. of water) and averaged 139 mm. of water.

In 43 determinations the total protein was normal in all fluids (exclusive of those contaminated by blood) irrespective of the presence of headache with the exception of 1 case which showed a normal value at a subsequent puncture.

In 10 instances, the amount of sugar present in the spinal fluid was within the normal range irrespective of the presence of headache.

In 7 fluids the chloride content was normal.

In all cases (44) the Ross-Jones and Pandy tests and colloidal gold reaction were normal except when the spinal fluid was contaminated with blood subsequent to hemorrhage at the point of puncture.

The cell count was normal (less than 6 cells per c.mm.) in all but 2 cases which had 6 and 11 cells per c.mm., respectively (bloody fluids excluded).

In all of the fluids the Wassermann (or Kahn) test was negative.

The authors observed no significant abnormality of, or consistent deviation from, the normal cerebrospinal fluid pressure in 44 cases of migraine. The total protein content, cytology and serology of the cerebrospinal fluid were normal. Any significant abnormality of the cerebrospinal fluid renders doubtful a diagnosis of migraine.

LYMPHOPATHIS VENEREUM, Its Relation to Rectal Stricture, Vander Veer, J. B., Cormia, F. E., and Ullery, J. C. Am. J. M. Sc. **190**: 128, 1935.

Of 47 cases of lymphopathia venereum infection here reported, 26 presented inguinal adenopathy and 21 rectal stricture. One case was complicated by esthiomene.

Lymphopathia venereum occurs with much greater frequency in the colored race.

Reading the Frei test after the fourth day is recommended.

The possibility of a diminution or absence of the Frei reaction in long-standing cases is considered.

All patients with inguinal adenopathy of obscure etiology and those with rectal stricture should be tested with Frei antigen.

Cases presenting perirectal abscesses, fistulae in ano, or obscure pelvic infections should also be tested to eliminate the possibility of a lymphopathia venereum infection as an etiologic factor.

There is no specific treatment for the disease. Various methods have been considered.

TRICHINOSIS, Diagnosis of, With Special Reference to Skin and Precipitin Tests, Spink, W. W., and Augustine, D. L. J. A. M. A. **104**: 1801, 1935.

Thirty-five sporadic cases of trichinosis occurring in and around Boston during the past three years were analyzed.

The most reliable diagnostic clinical aid in these cases was the presence of an eosinophilia.

The skin test usually becomes positive about the seventeenth day of the infection, and the precipitin test usually at the end of the fourth week. These tests are of especial diagnostic aid in the early stages of the disease, when they are first negative and later become positive. Mild, sporadic and chronic cases of trichinosis were often detected only by these tests.

Other laboratory procedures, such as searching for the parasite in the stools, blood and spinal fluid, are time consuming, and the larvae are only rarely found.

WASSERMANN-FASTNESS of Spinal Fluid in Treated Neurosyphilis, Goodman, M. J., and Moore, J. E. Arch. Int. Med. **55**: 827, 1935.

A study is presented of the incidence of clinical progression or relapse after prolonged treatment in 212 patients with neurosyphilis.

A comparison is made between two groups: 95 patients for whom the reaction of the spinal fluid remained persistently positive and 117 for whom the positive reaction was reversed by treatment.

Considering the groups as a whole, clinical progression occurred in 22 per cent of the Wassermann-fast patients and in only 7 per cent of those with reversed reactions.

Limiting the study to patients treated for two or more years, subsequent progression occurred in 12.5 per cent of the Wassermann-fast group and in 4.8 per cent of the group with reversed reactions.

Progression or relapse is more common in patients with parenchymatous neurosyphilis (tabes and dementia paralytica) than in those with nonparenchymatous types of neuraxis involvement.

Even in a patient with apparently nonparenchymatous neurosyphilis, the subsequent relapse, if one occurs, is likely to be dementia paralytica in type.

Of 31 patients in this series treated at some time with induced fever therapy (chiefly malaria), subsequent progression or relapse occurred in only 2.

While there is a more definite relationship between the clinical outcome and the reaction of the spinal fluid in neurosyphilis than between the reversal or the fastness of the Wassermann reaction of the blood in various forms of late syphilis not involving the nervous system, a persistently positive reaction of the spinal fluid does not indicate the inevitability of subsequent progresson or of relapse; nor can the rate or completeness of reversal of the reaction be used as the sole guide to the optimum duration of treatment in cases of neurosyphilis.

GLYCOSURIA, Factors Affecting the Appearance and Duration of, Robinson, C. S., Derivaux, R. C., and Hewell, B. Am. J. M. Sc. **189:** 795, 1935.

A study of the relationships between hyperglycemia and glycosuria after the intravenous injection of glucose indicates that the excretion of sugar may be explained on the basis of the suggested participation of phosphorylation in the resorption of glucose from the glomerular filtrate.

TUBERCULOSIS: Pathological Significance of the Leukocyte Reaction in: Leukocytic Index With a Calculator to Facilitate Computation, Crawford, A. M. Am. Rev. Tubere. **31:** 611, 1935.

A calculator has been devised for quickly and accurately determining the $\frac{N\%}{L\%}$ ratio value and for supplying the equivalent values for an abnormal elevation of monocytes and for abnormal total cell counts. This calculator is shown below.

From these values the leucocytic index is readily obtained for any given leucocytic picture.

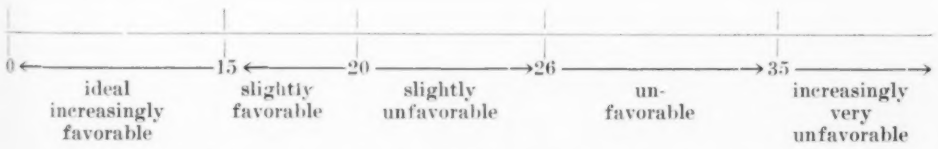
The leucocytic index is obtained by adding:

a: The $\frac{N\%}{L\%}$ ratio value

b: The value of abnormal monocyte percentages

c: The value of abnormal total cell counts

Interpretation of the index is given in terms of a favorable or unfavorable type of pathologic activity as follows:



The leucocytic index is suggested as a short cut to correct interpretation of the leucocytic reaction in terms of favorable or unfavorable pathologic activity as evaluated by Medlar. It is only a conventional short-hand symbol intended to represent the variation of the significant components of the leucocytic picture. The degree of pathologic activity having been determined by the index, it is urged that the components of leucocytic formulas be studied in order to get a complete understanding of the phase of the tuberculous process with which we are dealing. Medlar's conception of the significance of the rôle of each of the cell-types in the pathogenesis of the disease may thus be readily and accurately applied and his conception of the pathologic status of the tuberculeus process determined.

The Journal of Laboratory and Clinical Medicine

VOL. 21 DECEMBER, 1935 NO. 3

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Richmond, Va.

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Entered at the Post Office at St. Louis, Mo., as Second Class Matter

EDITORIALS

Thoughts on the Education of the Public

WITHOUT doubt the greatest obstacle to advance in the fight against disease—to use a hackneyed figure and a frayed and stereotyped phrase—is public ignorance combined with credulity. Were it not for these the path of the quacks, the peddler of patent pills and lotions and, it must be said, the careless and haphazard physician, would be hard indeed.

So obvious has this been that for lo! these many years physicians as represented by national, state, and local organizations have striven valiantly to educate the public through the dissemination of information concerning disease, its recognition, its management, and its control.

The methods used have been varied and, with the advent of the radio, broadcasts along the lines of health-programs have come into being with increasing frequency.

Despite the time, effort, and money expended the results, however, would seem in some degree disappointing, and it is of some interest to speculate

upon the probable or, at all events, theoretical reasons why this should be so.

Is it because the public does not wish to be educated? Hardly, for popular texts on health and allied subjects seem to be enjoying a marked approval.

Is it because the methods used are faulty or their application is inappropriate or requiring a different handling or a different touch? The problem presents many difficulties, chiefly, perhaps, because it is extremely difficult to ascertain with any degree of approximate exactitude the proportion of the population who read popular articles on health or listen to "health broadcasts" and the degree to which they are influenced by either or both.

It seems fair to wonder if, perhaps, the earnest seeker after such information in the columns of his favorite newspaper may not experience some slight confusion of ideas if he happens to note or be aware of the fact that the same fount of medical knowledge which pours forth information in a happy and popular vein, may also, if the day and hour be appropriately chosen, be heard spouting in even a happier vein (for the words of the radio are golden words, indeed) on the merits of some patent remedy at so much per spout.

But perhaps the broadcast comes with the accolade of some medical organization of standing and repute—what then? Simply that, perhaps from some private and commercial station or in the pages of varied publications come such ominous descriptions of the "medical trust" that even then the program must penetrate a murky mist of distrust and disbelief before it can begin to educate or inform.

It is not at all unlikely that the first reaction excited in the mind of the listener might be to the effect: Who is this fellow, anyway? Never heard of him! I wonder what my own doctor would say about that?

The answer to that possibility would logically seem to be the County Medical Society broadcast where the voice is familiar, the reputation known.

If we are to be candid, however, it must be admitted that many such programs fail lamentably of the purpose for reasons not always clear or obvious. Sometimes because the subjects chosen are trite and stereotyped; sometimes because the manner in which they are presented smacks of the textbook and is devoid of popular appeal; and sometimes because the speakers are chosen, not for their aptitude for the purpose, but with the idea of giving as many members of the Society as possible their little moment of pseudo-importance and acclaim.

It is the belief of the present deponent, who is, indeed, rather firmly convinced of the validity of the contention, that the disproportion between the efforts expended and the results evident in this matter of the education of the public are very largely based upon the truth expressed in the old, old adage which concerns the horse led to the trough.

Without doubt there is a public thirst for information in matters medical. Equally without doubt, there is a time and place when that desire is acute, when such information is not listened to simply as an unavoidable interim—happily brief—between orchestras, but when the entire attention of

the listener is focused on the problem, when, indeed, he goes out of his way in search of such information.

It may conceivably be somewhat difficult to interest an athlete in the mysteries of what is popularly known as "rheumatism." But this same subject is a matter of intense interest to the patient with joint-pains, just as food and its properties are of great interest to the woman eager to keep her girlish figure (overlooking the fact that she has become an older, if not wiser, matron), and of no interest (though it should be) to the individual who gets along nicely on three hearty meals a day, not to mention a nibble or two between and a little snack before going to bed.

Every patient wants to know, among other things, what is the matter with him, how he got that way, and why it makes him feel the way he does.

He may never put such questions articulately but the desire to know is there. And, if he is fortunate enough to encounter in his physician one who is able and willing to tell him—he will listen and try his darnedest to absorb the information he receives. And if, in this way, he learns only one thing at a time, at least he *learns* that and thus may become a center for its further dissemination.

And his physician knows, too, that someone is actually listening to what he has to say, not languidly, not inattentively, not perforce, but eagerly and avidly.

And it is the further belief of this deponent that the information given in the doctor's office to those who are anxious to acquire it will in the long run do more for the education of the public in matters medical than the set programs all the audience of which are certainly not equally interested in hearing them.

And so, having stated his belief that the best and most appropriate place for the education of the public is the doctor's office, and in so proclaiming having suggested that the physician ask himself if he is fulfilling to its greatest degree what may well be regarded as an important function reacting to the benefit of physician and patient alike; and, if not—why not?—further this deponent sayeth not.

—*R. A. K.*

Automatic Euthanasia

IT HAS been many years since William Osler suggested humorously that it might be well to terminate life at a time when it is no longer useful and becomes a burden to the aging individual. This was meat for the press of the time, and was therefore widely publicized. Cartoons lampooned and ladies' clubs debated. Theologians objected, moralists argued, and biologists produced statistics.

The furor died down, the impracticability of such a procedure being generally agreed upon, and "euthanasia" persisted as a household word, its meaning well understood.

But have we done with euthanasia? The arbitrarily controlled arrangement implied in the name is, of course, not to be considered. But is NATURE herself slowly and quietly arranging things in accordance with the suggestion made by Sir William?

Overpopulation scarcely seems a pressing problem at the present. There is so much of the world still uninhabited, and at the same time habitable. But overpopulation is not a world problem. It is usually one of a community, a country, or a geographic area or unit. War, famine, disease, and pestilence have prevented saturation in the past.

What is the present situation? We are faced with an economic problem in which those who have passed a certain age find it difficult to procure for themselves an adequate remuneration for services rendered. This is in essence a young man's world. The vast majority of aged individuals have failed to provide for their later years. They become burdens, if not to themselves, then to their children or the community. Even the efficient person past middle life finds it difficult to hold his position, much more so, to procure one.

The writer not long ago saw a laborer in his middle forties who, surprisingly, dyed his hair. Upon questioning he explained that he was prematurely gray, and that as long as his hair was gray he had found it practically impossible to procure a job.

For the individual the ideal arrangement on this earth would be a proper provision for comfortable old age, free from worry, for everybody. Possibly the old-age pensions recently inaugurated will help to this end, but at best they can provide little more than an escape from pauperism or dependency. Individualistically we cannot but hope for a comfortable old age with reasonable freedom from care. Biologically, however, there appear to be definite trends which promise to defeat the accomplishment of this objective.

The decline in infant mortality has increased the average duration of life. It has increased the population many fold. According to estimates, if there were no wars or epidemics, the present rate of increase would result in complete saturation of the world within one hundred years. At that time every tillable acre would be needed to provide sustenance, assuming there occur no new discoveries in chemistry to provide synthetic foods or otherwise simplify the problems of nutrition.

Assuming that such an eventuality is not desirable, what steps are likely to circumvent it? Birth control and the elimination of the unfit, preferably through sterilization and eugenic measures, appear ideal. Since, however, they interfere either with personal privilege or the ambitions of nations they will be slow indeed in gaining general acceptance and popularity.

War and disease, especially epidemic disease, remain as the most effective measures of combating the population increase which has resulted from the saving by science of infant and child life. We might add the automobile.

There may be another factor, of tantamount importance. We speak with regret of the good old days in which life was no such bustle, hurry, and confusion as it now is. We recall when competition was not so keen—when there was still time for the amenities—when life, though less speedy, was

enjoyed more in the living of it. Today's life of competition is one of stress and strain, drive and fatigue, and, all too often an early demise. Competitive effort and modern ways of living make it so. There is no escape, if one would achieve what is known as success.

Longevity of the individual has not been increased. Indeed there is evidence of an increased incidence of the degenerative diseases of middle life and the advancing years. If the strain of the modern day continues, there appears every probability that among those who reach maturity, the average life expectancy will be distinctly shorter than it has been. Men will continue to burn themselves out—to "kill themselves with overwork." And nothing that science can do will prevent it as long as civilization continues at its pace. An entire revamping of the manner of living of the whole world would be necessary. But with continued population increase, with every increasing competition, this is almost an impossibility.

Our great-grandfathers, pioneers and the like, died chiefly from the action of extrinsic factors—factors external to the body itself. Such were bullets, animals, bacteria, poisons, extreme heat or cold, starvation, injury and the like. Today, while these lethal factors continue active, we must add important contributory causes, intrinsic in origin. Other things being equal, the low speed engine will outlast the high speed machine.

All of which is little more than saying that modern life is a man-killing process.

Can we take exception to this? Is it really objectionable? To the individual, yes, very much so. Each and every one of us is imbued with the desire to live on and on. Biologically, there might be room for argument.

At what stage is man's function on earth completed? Warthin recognized three phases: (a) preparation for independent existence, which covers the period from birth through adolescence; (b) a period of twenty or more years during which the chief biologic function is the reproduction of the species; and (c) a period in which man protects his progeny until the latter is ready for independent existence. After the third phase the cycle is completed and man's function is terminated. There is no longer need for continued existence of the individual. One might argue that mental activity continues to grow or improve even after general physical involution has begun. This is probably true, and represents the only important reason for continued existence of the individual (a minority of individuals) after the biologic need for his living has ceased.

But this is of little interest to NATURE.

We are faced, therefore, with a situation in which we must recognize that NATURE may be quietly but inexorably exercising that function which Osler once suggested as possibly desirable. As individuals we cannot like it, but in the abstract we must admit that it is a logical counteractant to man's folly in persisting, with little reason, in his efforts to overpopulate the earth.

—*W. T. V.*